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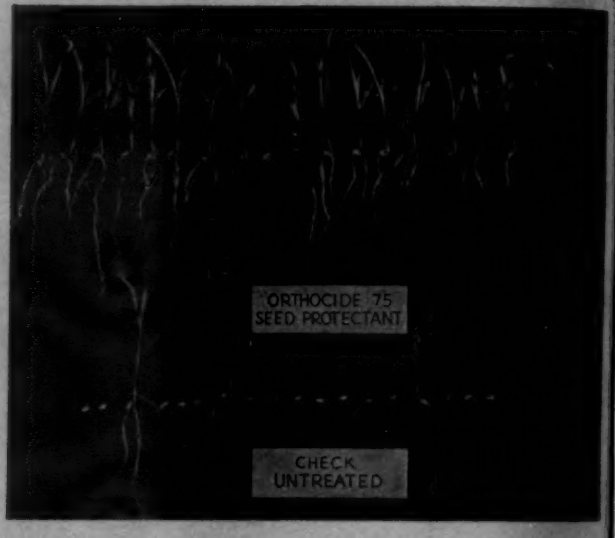

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FRED REUEL JONES, 1884–1956

E. W. Hanson and J. G. Dickson

Fred Reuel Jones, outstanding legume pathologist, died at Madison, Wisconsin April 5, 1956. He was internationally known for his work on the diseases of forage legumes.

The eldest of 3 children of John Henry and Matilda Beaman Jones, Fred was born June 9, 1884, on an apple farm in the rugged granite hills near Mercer, Maine. A mile down the hill from his home was the 1-room school with its excellent teacher who guided him through the elementary grades with great thoroughness. An illness of several years, during which he was tutored by a great aunt, a retired school teacher, made him late in his secondary education, which he obtained at Kent's Hill Seminary. Here he had opportunity to study Greek, Latin, and other classics which he thoroughly enjoyed and was to cultivate the rest of his days. He graduated from this school in the spring of 1905 and enrolled at Bates College at Lewiston, Maine, in the fall of the same year. At his father's death in 1907, the responsibility of managing the farm and its orchards fell to Fred, and it was necessary for him to return home. His formal education was not resumed until the autumn of 1910, this time at the University of Maine, where he received a Bachelor of Science degree in Horticulture on June 12, 1912.

Fred came to Wisconsin early in 1913 while on a tour of eastern and midwestern universities. During the visit he met Dr. L. R. Jones, then Chairman of the Department of Plant Pathology, and was greatly impressed by him as a teacher and naturalist. A few days later he enrolled at Wisconsin, where he received the M. S. degree in 1914 and the Ph.D. degree in 1917. His long association with the U. S. Department of Agriculture began in 1916, when he became a collaborator in what was then the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry. He joined the staff of the Department as a full-time employee on April 1, 1917, under the able leadership of Dr. W. A. Orton, and continued in the Department until September 30, 1950. Except for a few months during 1917-1918 when he spent some time in Washington, D. C., and at the U. S. Plant Introduction Station at Chico, California, he was located at Madison, Wisconsin. After his retirement from Government Service, he continued his work at the University of Wisconsin until the autumn of 1955 when he suffered a brain metastasis and his health declined rather rapidly.

His first research was done while he was still an undergraduate at the University of Maine. Here he completed a B.S. thesis on the development and extent of the roots of apple trees. His first study in plant pathology was on apple scab. Having observed orchard diseases at first hand from boyhood, it was natural that he should devote his first attention to one of these.

FRED REUEL JONES
1884–1956

The title of his M. S. thesis was, "Is the Venturia on the Wisconsin wild crab, *Pyrus coronaria*, identical with the *Venturia inaequalis* on the cultivated apple?"

His interest in the diseases of forage crops began in 1914 when he initiated a study of alfalfa and clover leaf spots: a critical and extensive work which did much to clarify the understanding of the etiology, symptoms, development, and other aspects of these diseases. He continued to work with legumes the rest of his life, spending most of his time on alfalfa diseases except for a period following World War I when he gave major attention to diseases of canning peas.

From the end of 1917 to the early part of 1920, Dr. Jones concentrated on the crownwart disease of alfalfa, and it was in connection with this malady that he was stationed at Chico, California, during the first months of 1918. During 1919 and 1920, he also studied the effect of soil temperature on the development of nodules on the roots of legumes. Beginning in 1918, but especially from 1920 to 1925, he investigated diseases of canning peas, particularly root rots

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wilts, and anthracnose. This work, done in cooperation with Charles Drechsler, R. E. Vaughan, M. B. Linford, and others, was outstanding, contributing much basic information, and making possible the subsequent development of varieties resistant to wilt.

Dr. Jones was best known for his researches on bacterial wilt of alfalfa, first observed by him in September, 1924, near Monroe, Wisconsin. He published the first report of this disease in 1925 and subsequently wrote at least 11 papers treating all of the important aspects of the problem. In 1930 he described a procedure whereby it became possible to compare alfalfa varieties for resistance to wilt in the space of a single year, thereby avoiding the delay and climatic hazards inevitable in a field test. This procedure permitted not only an evaluation of the degree of resistance in varieties but also the selection of resistant plants from which resistant strains could be developed. This lead to cooperative work with geneticists and plant breeders and the development of varieties resistant to wilt. He was a source of information, techniques, and cultures of the pathogen for researchers in all parts of the world and was an international authority on this disease.

Among his other contributions on alfalfa were his reports of resistance to common leaf spot (incited by *Pseudopeziza medicaginis* (Lib.) Sacc.) in 1941 and to yellow leaf blotch (incited by *P. jonesii* (Fckl.) Nannf.) in 1949. In 1953 he published a unique method for the rapid measurement of resistance to common leaf spot. His studies on winter injury, longevity, seed setting, the relation of heat and drought to foliage yellowing and floral injury, and various morphological and physiological characters of alfalfa were extensive.

Several of his studies of sweetclover diseases deserve special mention. He was the first to report the occurrence in the United States of stem canker, also called "Gooseneck" (incited by *Ascochyta caulicola* Laub.). In 1937 he discovered the perithecial stage of *Cercospora davisii* Ell. & Ev. and after a critical study named it *Mycosphaerella davisii* sp. nov. He clarified the confusion as to the correct name of the conidial stage of *Mycosphaerella lethalis* Stone, reported resistance to Phytophthora root rot, and showed that *Ascochyta caulicola*, *Cercospora davisii*, *Leptosphaeria pratensis* Sacc. & Briard, and *Mycosphaerella lethalis* are seed-borne.

He was very interested in mycorrhizal fungi and devoted some time to soybean diseases. In 1946 he reported systemic infection of downy mildew (*Peronospora manchurica* (Naoum.) Syd.) in soybean.

Dr. Jones' greatest devotion was to his work, but he had many other interests and avocations. He loved the out-of-doors in all of its aspects and was very fond of camping, canoeing, skating, skiing, and hiking. He was a good photographer and one of the most active members of the Madison Geological Society. Music and art, especially etchings and Japanese prints, interested him, and he was particularly fond of good literature, both prose and poetry, which he studied critically and discussed with fellow members of the several literary groups to which he belonged. He enjoyed young people and gave many hours of his time to Boy Scout activities and to students.

Fred Jones was an intellectual with a brilliant and discriminating mind, a philosopher, and an extraordinary scholar, but also he was a likeable, understanding, patient, and kind person. Selfishness was never a part of his nature. He was a man of integrity and high ideals, a mild-mannered individual who never forced his ideas, desires, or actions on anyone else, but also insisted on freedom of thought and action for himself. He was meticulous and thorough in his work and precise and analytical in his thinking.

He was a fellow of the American Association for the Advancement of Science and a member of many other scientific and scholarly organizations. Among these were the American Phytopathological Society; the Indian Phytopathological Society; the Mycological Society of America; the Botanical Society of America; the American Society of Naturalists; the American Society of Agronomy; the Wisconsin Academy of Sciences, Arts and Letters; the Madison Geological Society; Sigma Xi; Alpha Zeta; and Gamma Alpha.

He was married May 26, 1921 to Edith K. Seymour, whose charm, understanding, and unselfish consideration for others, combined with Fred's humor and good conversation, endeared the whole family to friends and associates. The Jones' home was always open to all who would come. Besides his wife, he is survived by 1 daughter, Phoebe Ellen (Mrs. Franz Samelson); 2 sons, Francis Seymour and Fred Reuel, Jr.; 1 sister; 1 brother; and 4 grandchildren.

Fred's life was a fulfillment of many of his dreams. He had a happy home, a productive and satisfying career, and a host of friends. He inspired countless students to a deeper appreciation of the meaning of life and the potentialities which science has in uplifting man and in improving his existence.

RELATIONSHIP OF INJURY INDEXES AND FUMIGATION FLUORIDE LEVELS[1]

D. F. Adams, C. G. Shaw, and W. D. Yerkes, Jr.[2]

SUMMARY

Fumigation of 2 varieties of gladiolus, Ethel Cave Cole and Snow Princess, at 3 fumigation levels for different exposure times indicated a direct relationship between the fumigation exposure factors and the foliar injury produced. The data further indicated that Ethel Cave Cole is slightly more sensitive to hydrogen fluoride than is Snow Princess.

Needles of *Pinus ponderosa* were many times more sensitive to fluoride fumigation in the immature partially elongated state, than in the more mature, completely elongated stage. Fumigation of seedlings with normally expanded needles at 3 intervals during the growing season substantiates the observation in the field that the older the needles, the more resistant they become to atmospheric fluorides, even though the older needles may have higher foliar fluoride levels.

The statistical relationships between injury indexes and exposure factors for 2 varieties of gladiolus and for *Pinus ponderosa* with needles in 2 stages of maturity are in excess of that required for confidence at the 1 per cent level.

Contamination of the atmosphere by gaseous fluorides arising from industrial operations has produced detrimental effects upon vegetation in many areas (8, 9). Field observations of foliar injury have been supplemented by observations on healthy plants subjected to atmospheres containing hydrogen fluoride. Early controlled experiments involving the exposure of vegetation to hydrogen fluoride gas were conducted in the concentration range of 0.5 to 10 ppm for periods of several hours (5, 6, 10). Analyses of the atmosphere in several industrial areas have shown atmospheric concentrations of fluoride to be in the order of a few tenths to approximately 20 parts per billion (1, 4). These analyses were based on 4-hour samples. Consequently, the authors have exposed plants to hydrogen fluoride in this concentration range.

Johnson et al (7) have shown that different varieties of gladiolus vary in susceptibility to atmospheric fluoric effluents under field conditions. This is a report of experiments designed to compare the extent of foliar injury on the gladiolus (*Gladiolus hortulanus* Bailey) varieties, Ethel Cave Cole and Snow Princess, and on ponderosa pine (*Pinus ponderosa* Laws.) at different stages of maturity when continuously exposed for varying lengths of time to 3 controlled levels of fumigation.

SYMPTOMS.—Fluorine damage to gladiolus has been described as "a burned appearance of the leaves extending from the tips downward or along the margins and across the leaf lamina. The color is usually dark brown with scattered areas or patches of light tan. There is a considerable variation between varieties, some having the tan, others the dark brown prevailing. The affected areas are irregular in size and shape and occur parallel with the veins. The veins as well as the interveinal tissue become burned. . . . The outstanding characteristic is the light tan discoloration with a dark brown margin" (7).

Fluorine damage to ponderosa pine appears as a light-brown to reddish-brown discoloration extending from the tips of the needles downward. This discoloration may be either continuous (Fig. 1, A) or interrupted by 1 or more narrow darker red-brown bands (Fig. 1, B). "Banding" usually occurs as a result of intermittent rather than continuous exposure to fluorides. To produce the "banding" type of injury, intermittent fumigations should be separated by time intervals of 3–5 days, to allow complete development of the damage caused by each previous exposure. Frequently the banding may be noted also at the interface between necrotic and healthy tissue.

Typical fluoride injury might be confused with winter damage, for they appear similar upon casual examination; however, careful inspection will allow differentiation in most cases. Winter damage occurs during the winter or early spring on needles produced during previous growing seasons, particularly on the older needles. Symptom development is slow; the injury initially is indicated only by a slight chlorosis. The disease becomes readily apparent only with the advent of warm weather in the spring. Fluoride injury occurs most frequently during the growing season. Indeed, injury characteristically occurs during June, when the needles are elongating, and during July and early August. The needles apparently become increasingly resistant as they mature, with the result that fully matured needles and needles 1 or more years old are injured only if exposed to much higher concentrations than are necessary to injure immature needles. Symptoms of fluoride injury appear suddenly within a few days after exposure.

Winter damage and fluoride injury also produce different patterns of injury within a stand. Winter

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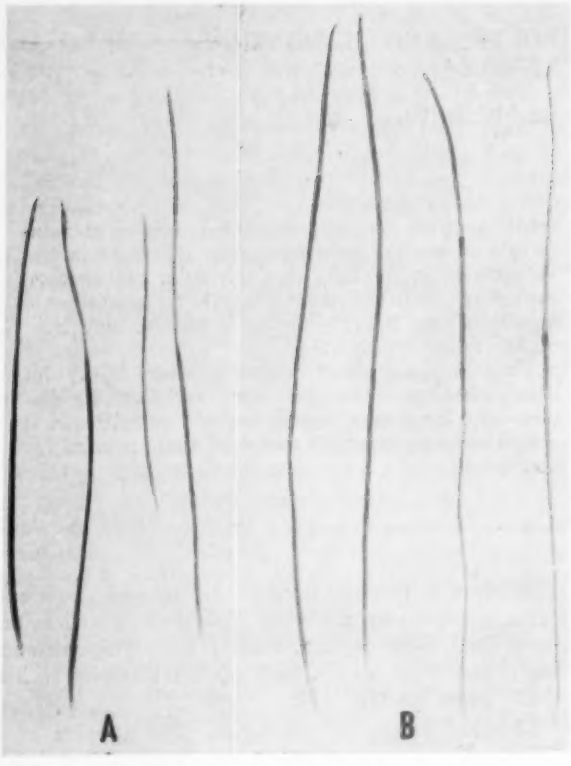

FIG. 1. Fluoride injury to ponderosa pine. A) Injury typical of a single exposure. B) Injury resulting from successive exposures at intervals.

damage often is most severe on the sides of trees or stands facing warm winds and the sun. There is no such correlation in the case of fluoride injury. Despite these differences, however, chemical analyses of the affected tissue should be obtained as confirmatory evidence in all cases of suspected fluoride injury.

EXPERIMENTAL PROCEDURES.—*Fumigation equipment.*—Fumigation greenhouses, constructed of Fiberpane plastic, were used as enclosures in which varying concentrations of hydrogen fluoride were developed and maintained. The air in the greenhouses was exchanged approximately once every 2 minutes. Known quantities of a mixture of anhydrous hydrogen fluoride and nitrogen were introduced under constant pressure into the incoming stream of washed air. The contaminated air was exhausted through a counter-current spray washer to prevent both recirculation of fluoride-containing air and contamination of vegetation in the vicinity of the fumigation chambers. The uniformity of air flow through the chambers was visually checked with paper streamers hung at the average foliage level throughout the greenhouses. The incoming airflow baffles were adjusted so that an evenness of motion was obtained. The primary features of the fumigation equipment are illustrated in Figure 2.

Analytical methods.—Air samples were obtained by continuous withdrawal from the fumigation chamber during each run. Samples represented either 4- or 8-hour composites, depending upon the concentration range within the chamber. The air samples were passed through scrubbing towers which have been described by Adams et al (1). The fluoride content of the absorption liquid was determined by direct thorium nitrate titration (3).

Foliage samples were collected 3 days after completion of each fumigation run, placed in 1-quart containers with 4 g of Fischer "low in fluorine" lime, and shaken. The samples were dried in an oven for 24

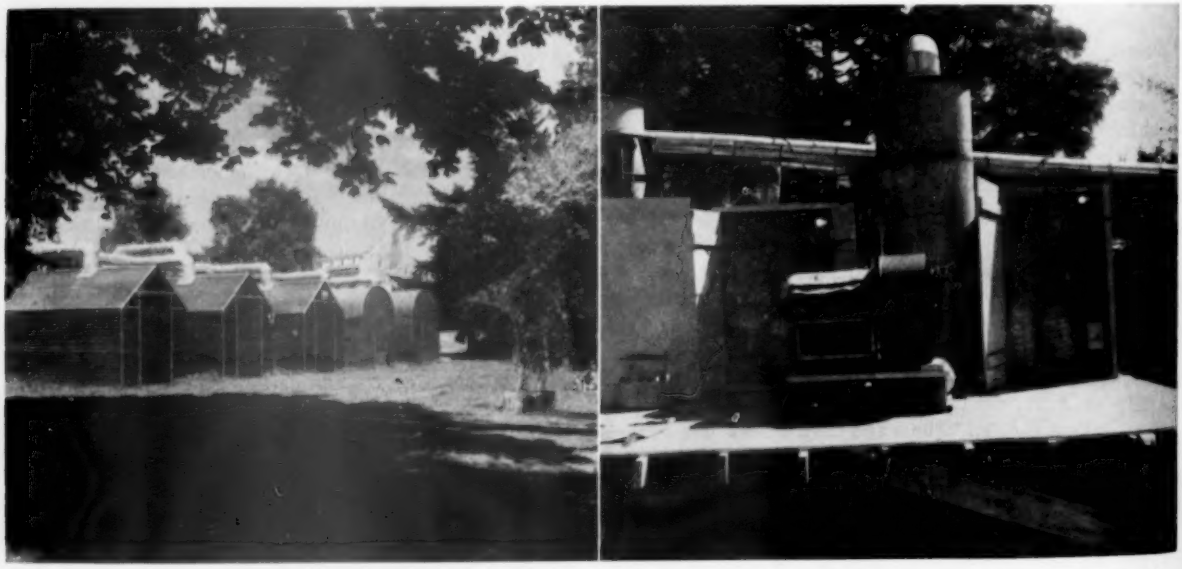

FIG. 2. Left: the experimental fumigation greenhouses. Right: The regulatory equipment used for controlling the atmosphere within the greenhouses during fumigation.

hours at 70°C and the dry weight determined. The entire sample, after drying, was placed in an inconel crucible and a slurry made with water to assure penetration of the alkali into the foliage tissue. The sample was then dried on a hot plate under an infrared lamp and ashed in a muffle furnace for 30 minutes at 600°C. A 1-g sample of the ash was distilled from perchloric acid, and an aliquot of the distillate was titrated with thorium nitrate (3).

Fumigation specimens.—Ethel Cave Cole and Snow Princess gladioli were planted in 1-gal. cans, 4–5 corms per can. The soil used was of uniform local origin. The gladioli used in these experiments had all developed their fourth true leaf, and an occasional plant showed a fifth leaf.

Ponderosa pine seedlings, approximately 5 years old, were planted in 5-gal. cans in local soil. Three to 5 seedlings were grown in each container. All plants were watered daily throughout the progress of the experiment. The ponderosa pine trees were divided into 2 equal groups in early spring. One group was placed in a greenhouse in early May for forcing. The needles of these trees were completely expanded by early June, at which time the trees were placed outside for a week to harden before fumigation was initiated. These trees are termed "forced" in the following discussion. A second group of trees was placed outdoors; their needles expanded at the normal rate. The needles had expanded less than 50 per cent of their total elongation by June 26, the completion date of the fumigation tests. These trees are termed "normal" in this discussion. This procedure provided specimens in 2 states of maturity for fumigation by mid-June.

Fumigation procedure.—Plants were exposed to 3 atmospheric levels of hydrogen fluoride (HF) fumigant: 0.7, 1.2, and 12 parts per billion. Five pots each of Ethel Cave Cole and Snow Princess gladioli and of "normal" and "forced" ponderosa pines were placed in the fumigation chamber at the start of each exposure. One pot of each was removed at the end of each 24-hour interval over a continuous exposure period of 120 hours. Three days after termination of the fumigation sequence, during which the injury pattern became completely developed, each gladiolus leaf was measured to determine the proportion of damage to total length of the leaf. The average injury index for each pot of gladiolus was determined from the injury indexes calculated for each leaf. The injury index is 100 times the ratio of damaged length to total leaf length (7). Measurements also were made on ponderosa pine to determine the total number of current year's needles, the number of these needles exhibiting burn, the average length of needles, and the average length of needle burn. A pine injury index was calculated for each seedling, and the average injury index for each pot of ponderosa pine was then calculated (2).

The degree of exposure to hydrogen fluoride fumigant is expressed as an "exposure factor." This factor is defined as the sum of the product of the fumigation concentration expressed as parts per billion hydrogen fluoride times the length of exposure expressed as hours per day.

EXPERIMENTAL RESULTS. — *Gladiolus.* — The results of the gladiolus fumigations at 0.7, 1.2, and 12 parts per billion (ppb) hydrogen fluoride are given in Table 1. These data show the injury index for each variety at each exposure time and concentration. Each index is the average of measurements made on 16–20 leaves. Averaging the injury indexes for each variety at all fumigation levels and exposure times gave an index of 18.4 for Ethel Cave Cole and 17.5 for Snow Princess.

Correlation coefficients, relating injury indexes to the exposure factors, showed a degree of statistical relationship in excess of that required for confidence at the 1 per cent level.

Ponderosa pine.—The injuries produced at 2 concentration levels on "normal" and "forced" pine trees are compared in Table 2. The results indicate that the immature partially expanded needles were more susceptible to hydrogen fluoride than were the mature needles, thus confirming field observations.

The correlation coefficients (Table 2) relating the injury indexes to the exposure factors for needles of approximately 50 per cent and 100 per cent elongation showed a degree of statistical significance in excess of that required for confidence at the 1 per cent level. The injury indexes for "normal" needles of ponderosa pine with exposure factors above 576 were

TABLE 1.—*Comparative injury indexes of 2 varieties of gladiolus fumigated for different periods at 3 hydrogen fluoride levels*

Exposure time (hours)	Exposure factor	Injury indexes[a] of indicated variety	
		E. C. Cole	Snow Princess
Exposure 1: 12 ppb HF, June 11–16, 1951			
24	288	25.7	22.1
48	576	35.0	37.3
72	864	46.7	57.2
96	1152	52.9	44.6
120	1440	52.0	53.0
Exposure 2: 1.2 ppb HF, June 19–24, 1951			
24	28.8	1.9	0.0
48	57.6	6.1	6.7
72	86.4	6.9	5.7
96	115.2	15.4	13.7
120	144.0	20.7	15.3
Exposure 3: 0.7 ppb HF, June 26–July 1, 1951			
24	16.8	0.7	0.1
48	33.6	2.0	1.2
72	50.4	3.1	1.4
96	67.4	2.9	2.3
120	84.0	4.2	2.0

[a] The injury index is 100 times the ratio of injured length to total leaf length. The indexes are the averages for 19–30 leaves.

[b] Coefficients of correlation between injury indexes and exposure factors are as follows: Ethel Cave Cole, $r = +0.874$; Snow Princess, $r = +0.931$. Both show statistical relationships in excess of that required for confidence at the 1 per cent level.

TABLE 2.—*Comparative injury indexes of ponderosa pine fumigated for different periods at 2 hydrogen fluoride levels*

Exposure time (hours)	Exposure factor	Injury indexes of needles in indicated stage of maturity	
		"Normal" needles	"Forced" needles
Exposure 1: 12 ppb HF, June 11–16, 1951			
24	288	66.7	52.5
48	576	100	49.9
72	864	100	87.8
96	1152	100	100
120	1440	100	89.2
Exposure 2: 1.2 ppb HF, June 19–24, 1951			
24	28.8	0.5	0.0
48	57.6	1.0	0.4
72	86.4	4.6	1.4
96	115.2	33.2	5.5
120	144.0	34.0	5.0

[a] The total current season's needle length and length of needle injury was measured on 10 per cent of the burned needles; however, these measurements were obtained on at least 10 needles whenever possible, even though 10 per cent of the burned needles might be a lesser number. The injury index for each individual seedling is the percentage of total needles burned multiplied by the ratio of the average length of injured tissue to the average needle length of the needles measured.

[b] Coefficients of correlation between injury indexes and exposure factors are as follows: "Normal" needles, $r = +0.990$; "Forced" needles, $r = +0.933$. Both show a statistical relationship in excess of that required for confidence at the 1 per cent level.

omitted from consideration, since they were obviously completely injured by an exposure less than the total.

Three additional fumigation exposures were given "normal" ponderosa pine seedlings. Two replicated exposures on July 3, for 24 hours at average concentrations of 5.8 and 5.7 ppb HF, produced an average injury index of 40 at an average foliar fluoride level of 25 ppm. A second fumigation, begun on July 24, produced an average injury index of 5 at an average foliar fluoride level of 46 ppm after fumigation for a total of 134 hours at 5.4 ppb HF. A late-season fumigation, begun on September 8, was conducted for 96 hours at an average concentration of 5.7 ppb HF without producing any visible damage at an average foliar fluoride level of 60 ppm. This last fumigation was concluded before any visible injury was produced because of the onset of freezing weather.

DISCUSSION.—Whereas the correlation between exposure factors and injury indexes is extremely high, there are certain inconsistencies in the data that need comment. For example, Snow Princess gladiolus exhibited a lower injury index at 96 hours (exposure factor, 1152) than at 72 hours (exposure factor, 864) when fumigated at 12 ppb; both Snow Princess and Ethel Cave Cole exhibited less injury at exposure factors of 67.4 and 84.0 in the 0.7-ppb fumigation than at an exposure factor of 57.6 in the 1.2-ppb fumigation (Table 2). An exposure factor of 1152 gave an injury index of 100 on "forced" ponderosa pine, whereas an exposure factor of 1440 gave an injury index of 89.2 (Table 2).

The manner of development of the leaves of gladiolus (growth from the base) is such that each newly elongating leaf is enclosed by the older leaves to a greater extent than the next youngest leaf. Our observations indicate that only the unenclosed terminal portions of the leaves are subject to fluoride injury. If the youngest leaf of a plant is just emerging at the time of exposure, only 1 in. may be exposed, even though the total length of that leaf from the base of the plant may be 20 in. Such a leaf has a maximum potential injury index of $1/20 \times 100 = 5$. If, on the other hand, the youngest leaf is essentially fully elongated, it may be 30 in. in length with 10 in. exposed. Under such circumstances the maximum potential injury index is $10/30 \times 100 = 33.3$. This variation in development of the youngest leaf (or even the 2 youngest leaves) undoubtedly is responsible for some of the deviations from the direct relationship between exposure factors and gladiolus injury indexes indicated by the data as a whole. Injury indexes for gladiolus of from 45 to 55 indicate that essentially all the exposed leaf-length has been burned.

During our studies of fluoride injury, we have repeatedly noted differences in susceptibility not only among varieties but also from one individual to another within the same variety. If all individuals in a native stand or in seedlings grown at the same time in the nursery from the same seed source are considered to belong to the same "variety," this intravarietal difference is most striking in ponderosa pine. In the field, we have often observed 1 or 2 trees showing either no injury or very little, whereas the great majority of trees immediately adjacent had been either severely burned or killed. Similar observations have been made during controlled fumigations of ponderosa pine. In a group of seedlings subjected to the same fumigation, often 1 or 2 show relatively little burn, the rest being severely burned. The presence of all susceptible individuals in the group of "forced" ponderosa pine seedlings with an exposure factor of 1152 and the presence of a single slightly resistant individual in the group with an exposure factor of 1440 explains the apparent discrepancy of the injury indexes.

Variability in the susceptibility of different individuals of the same species or variety, correlated with the numbers of individual plants involved in each exposure in these experiments, and variability in development of the youngest leaf in the case of gladiolus may well account for the exceptions to the direct relationship between the fumigation exposure factors and the injury indexes. Despite these exceptions, the correlation between the exposure factors and the injury indexes is highly significant.

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INVESTIGATIONS ON THE FOOT AND ROOT PHASE OF SAFFLOWER RUST [1]

M. L. Schuster [2]

SUMMARY

Soil temperature affects the pathogenicity of soil- or seed-borne inocula of the safflower rust pathogen, *Puccinia carthami* Cda. The foot and root phase of the disease was favored by temperatures of 15°, 20°, and 25°C and hindered by temperatures of 30° and 35°. The amount of rust did not differ materially when soil moisture levels were 35, 60, or 80 per cent of the water-holding capacity. In the field, early planting favored disease development from soil-borne inocula. Under field conditions, teliospores survived for 12 but not for 21 months; when maintained in storage at about 5°C, infested safflower straw contained viable teliospores for at least 45 months. Variability in *P. carthami* was indicated but was not definitely determined, and differences were evident in safflower varietal reaction to infection by urediospores and teliospores. Seed treatment and side dressings of seed proved unsuccessful in preventing infection by soil-borne inocula. Acti-dione, at concentrations of 250 or 1000 ppm, decreased emergence, but the emerged seedlings remained free from rust.

A destructive foot and root disease of safflower (*Carthamus tinctorious* L.) caused by the rust fungus *Puccinia carthami* Cda. was first reported and produced experimentally in 1952 by Schuster and Christiansen (6) and has since been observed by others (1, 5). Comparatively little work has been done on the problems related to the influence of soil factors on the disease and its causal organism. The results of an investigation on variability of the pathogen and on methods of control pertinent to the foot and root phase of the disease are recorded in this paper.

Experimental studies.—*Effect of differences in soil temperature on the disease.*—This rust fungus is seed- and soil-borne and therefore lends itself to a study of the effects of such soil factors as temperature and moisture. The influence of soil temperature upon the disease was studied with the aid of temperature tanks heated by electrical heating coils that were thermostatically controlled to provide various soil temperatures ranging from 15° to 35°C at 5-degree intervals. Each treatment was replicated 4 times. Ten inoculated and 10 noninoculated 2-day-old safflower seedlings of the N-8 variety were transplanted into each culture can. The seedlings were submerged in a suspension of teliospores of *P. carthami* before transplanting, a method of inoculation that proved as effective as did seed inoculations.

During the winter, most of the experiments were

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conducted in a greenhouse with a fairly constant temperature of about 22°C. The effect of air temperature on soil temperature was minimized by keeping the culture cans covered until emergence of seedlings.

A soil mixture of 2 parts field soil, 1 part sand, and 1 part compost was steam sterilized, and 9 kg was added to each can. An attempt was made to maintain the moisture content at 19.6 per cent of the dry weight of the soil (35 per cent of moisture-holding capacity) by daily watering, since experimental evidence indicated that this moisture content maintained plant growth to good advantage and that the 60 and 80 per cent moisture levels had little differential effect on pathogenesis of the safflower rust fungus. To insure the least possible change of soil temperature during periodic waterings, water of the same temperature as the treatment was added to the respective cans.

The disease readings were made about 3 weeks after planting. It was noted that pycnia were evident as soon as 9 days after planting and that at the termination of the experiment the uredial stage of development was most common at the sites of infection. The data indicate that soil temperature definitely influences the incidence of this disease (Table 1). The lower temperatures (15°, 20°, and 25°C) proved favorable. At the higher temperatures (30° and 35°C), infection or rust development did not occur. This is somewhat in conformance with the results of Prasada and Chothia (4), who found no germination of teliospores at temperatures above 26°C in laboratory experiments in India. They found that only a few teliospores germinated at the 20°–22°C range, however, whereas our results indicate high germination (a large percentage of plants were infected) at 20°. Calvert et al (2), who conducted preliminary tests in 1954 with temperatures no higher than 20°C, also obtained infection from seed-borne inocula at this temperature. The discrepancy between laboratory germination tests and soil pathogenicity tests may be in the possible effect of soil constituents, in host effects on spore germination, or in variability in the rust organism. Unsuccessful attempts were made to obtain formation of sporidia by germination of teliospores in water, on seedlings in observation boxes, and on filter paper imbedded in the soil under the optimum temperature conditions reported by Prasada and Chothia (4). In a few instances, a fraction of 1 per cent of teliospores in water on slides germinated without sporidial formation.

In a preliminary test in which treatments were conducted in duplicate, results were at variance with the above soil-temperature data. At soil temperatures of 15°, 20°, 25°, 30°, and 35°C, the percentages of infective seedlings were 86, 76, 83, 60, and 0, respectively. In this preliminary test, tap water was added to the culture cans during periodic waterings. Whether the amount of water added decreased the soil temperatures of the 30°C cans for a period sufficiently long to allow germination was not determined; nevertheless, our 3 subsequent experiments indicated that at 30°C the rust fungus is nonpathogenic.

Effect of time of planting on disease incidence.—An attempt to relate the information on the effect of soil temperature on pathogenicity in greenhouse tests to safflower rust in the field was made by employing several dates of planting.

The safflower variety N-8 was planted at weekly intervals in the springs of 1952, 1953, 1954, and 1955 at the Scotts Bluff Experiment Station, Mitchell, in plots that had contained rusted safflower the previous year. Seed was sown with a hand planter set to operate at 1½- to 2-in. depths. The first planting (April 21) was made during the middle of the recommended planting time in the area. Soil temperatures at the 1½- to 2-in. depths were determined in 1953 at infrequent intervals with a standard mercury thermometer; in 1954 and 1955 the readings at the desired depth were recorded continuously by a soil thermograph. The soil was a Tripp very fine sandy loam; each planting date was in quadruplicate. Field experiments appeared to confirm controlled greenhouse tests on temperature effects on infection (Tables 2, 3). Safflower planted early in the spring, when low soil temperatures prevailed, showed a greater amount of infection than did that planted later, when the soil was warm. Little or no infection developed on late-planted safflower. As the season advanced, safflower plantings escaped infection from the soil-borne inocula

TABLE 1.—*Percentage of safflower plants affected by the root and foot phase of safflower rust when inoculated plants were grown at different soil temperatures in greenhouse experiments*

Year	Percentage[a] of infected plants at indicated soil temperatures (°C)				
	15°	20°	25°	30°	35°
1952	74	68	44	0	0
1952	58	58	7	0	0
1956	55	44	21	0	0

[a] Average of 4 replications.

TABLE 2.—*Incidence of root and foot phase of safflower rust in plants that were planted at different dates at the Scotts Bluff Experiment Station in plots infested with Puccinia carthami*

Date of planting	Percentage[a] of plants rusted			
	1952	1953	1954	1955
April 21	60	76	—	50
April 28	—	—	0	41
May 3	—	—	6	48
May 10	—	—	0	53
May 17	—	—	0	36
May 24	—	17	—	23
May 31	—	—	0	8
June 7	0.6	—	0	13
June 14	0	0	0	12
June 21	—	—	0	0
June 28	—	—	—	0
July 5	0	—	—	0
July 26	—	0	—	—

[a] Average of 4 replications. Dash indicates planting not made on that date.

TABLE 3.—*Mean weekly maximum and minimum soil temperatures at a depth of 1½–2 in. at the Scotts Bluff Experiment Station, Mitchell, Nebraska, in 1954 and 1955*

Week	Soil temperature[a] in degrees Centigrade					
	Mean		Minimum		Maximum	
	1954	1955	1954	1955	1954	1955
April 21-27	—	15	—	7	—	22
April 28-May 2	3	16	2	9	8	23
May 3-9	12	16	8	7	21	24
May 10-16	16	19	8	12	29	28
May 17-23[b]	19	18	8	10	35	26
May 24-30	15	16	7	9	30	22
May 31-June 6	17	19	6	13	33	26
June 7-13	20	16	9	10	29	21
June 14-20	22	18	15	12	28	23
June 21-27	25	23	18	16	35	31
June 28-July 4	27	24	16	16	30	32
July 5-11	28	28	22	18	31	38
July 12-18	26	28	20	18	34	37
July 19-25	—	27	—	19	—	33
July 26-Aug. 1	—	25	—	18	—	30

[a] Dash indicates not recorded.
[b] Because of the delayed emergence of seed planted the first 5 dates, the soil temperature for the May 17–May 23 period could be considered as applicable for the entire first 5-week period.

as well as from the teliospores borne on the seed.

Normally it requires about a week for plants to emerge; it is assumed from greenhouse experiments that infection occurs during this period (6). On the basis of this assumption, the soil temperature records for a 1-week period after planting are considered of major importance. Mean temperature was not the deciding factor in the field tests, since infection should have appeared where planting was as late as June 21 in 1954 and June 28 in 1955 (Table 3). The average mean temperatures in 1955 for the June 21–27 and June 28–July 4 periods were 23 and 24°C, respectively. Both temperatures are favorable for infection. It appears then that maximum temperatures are of primary importance in pathogenicity. This was true in the 1954 experiments in which infection developed only in the plants in plots seeded on the second planting date. Subsequent to that date, the maximum temperatures ranged from 28 to 35°C, which are too high for infection. During the week following the first planting, the temperature may have been too low for teliospore germination. This would be in accord with a report by Prasada and Chothia (4), who found no germination at 7–8°C. In the 1955 experiments, first emergence did not occur until May 23; hence, the first 5 planting dates can be considered as beginning on approximately May 17. The delayed emergence was due to dry soil; it did not rain until May 18. This may explain the relatively high percentage of infection at maximum temperatures of 28°C during the May 10–16 period in 1955. This also explains the relatively uniform amount of infection for the first 5 dates of planting in 1955.

Similar observations on the effect of maximum temperature were made in April–May, 1955. In 3 seed-treatment tests in the greenhouse, infection did not occur. Although the soil temperature during the greater period of the day remained at 22–23°C, the soil temperature would rise to 28–30°C during midday. Infection resulted in simultaneous tests in which soil temperature remained at 18°C.

Longevity of P. carthami.—From the date-of-planting experiments, it is quite evident that teliospores of *P. carthami* survive from harvest time in the autumn to early spring or for a period of about 6 months. Knowledge of the longevity of fungus in the soil may clarify some of the results obtained with the time-of-planting experiments and may be necessary in making control recommendations.

Investigations along these lines were conducted during the 1950–1955 period. Infested safflower straw was kept in wooden containers, either with or without soil, outdoors or in storage at 5°C. Containers were kept on the ground in Lincoln; at the Scotts Bluff Experiment Station they were sunk in the soil to simulate field conditions.

Viability of teliospores was determined by pathogenicity tests. The infested straw or the soil-and-straw mixture was added to pots, and 10 seeds of the N-8 variety were planted in each pot. The controls consisted of seed planted in field soil without infested straw. Disease readings were made 25 days after planting.

Infested safflower straw exposed to natural conditions contained viable teliospores for 12 months but not for 21 months (Table 4). These data demonstrate

TABLE 4.—*Longevity of teliospores of Puccinia carthami under various conditions as determined by pathogenicity tests in the greenhouse, 1950-1955 period*

Storage condition[a]	Location (Nebraska)	Age of inocula in months	Percentage of plants rusted
Infested straw in soil[b]	Mitchell	9	25
		12	40
		21	0[c]
	Lincoln	6	78
		12	4[c]
		21	0
Infested straw atop soil[b]	Lincoln	6	83
		21	0[c]
Noninfested soil check[b, d]	Mitchell and Lincoln	2-45	0
Infested straw at 5° C	Lincoln	2	17
		9	100
		12	6
		21	68
		24	51
		33	43
		45	10
Infested straw in soil at 5° C	Lincoln	6	14
		12	4
		24	13

[a] Five replicates of each treatment.
[b] Maintained outdoors.
[c] Experiments were duplicated.
[d] Includes checks for all experiments.

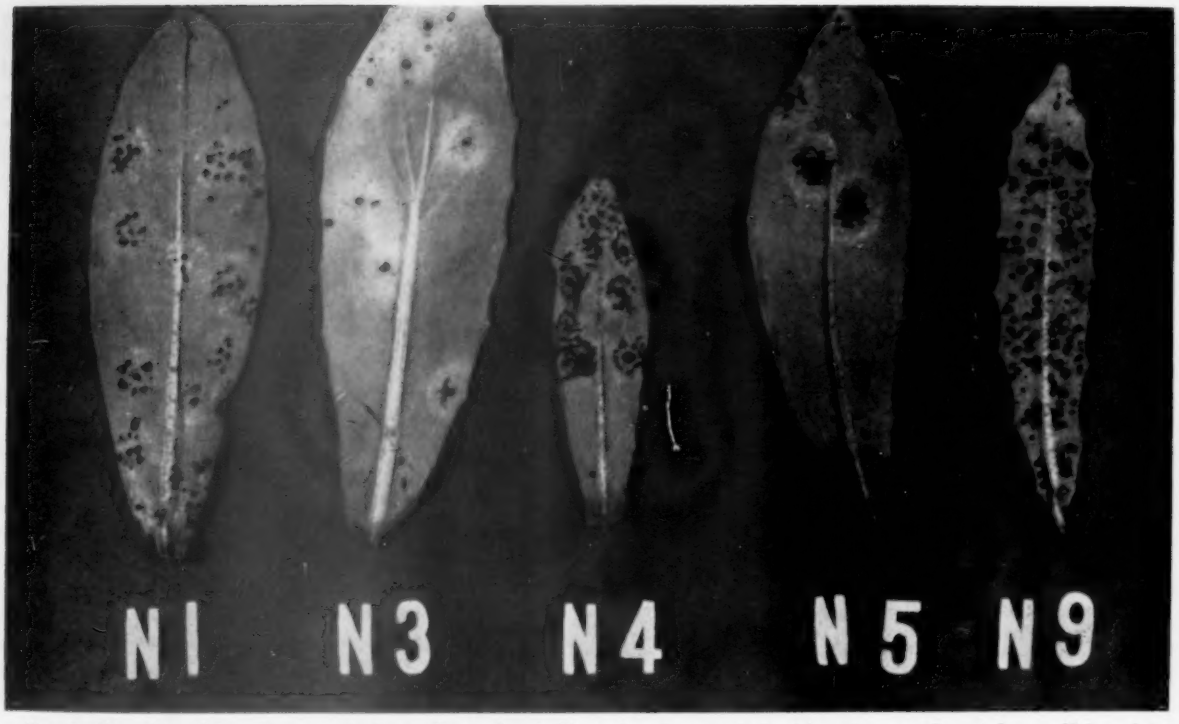

FIG. 1. Reaction of 5 safflower varieties to *P. carthami*. Note the characteristic chlorosis around pustules on N-3 variety.

that *P. carthami* is incapable of surviving 2 winters in the field in Nebraska, although a small percentage of teliospores remain viable for at least 45 months when stored at 5°C. Teliospores did not survive 21 months in the field regardless of whether the infested straw was kept on the surface or mixed in with the soil. Control of rust from soil-borne inocula may be accomplished by not planting safflower on the same field in successive years.

Control by seed and soil treatments.—Prevention of infection from soil-borne inocula by seed dressing with chemicals has not been promising. It has been reported that spore load may affect the degree of control by seed treatment (6).

Experiments were continued by the use of some newer fungicides as seed treatments as well as side dressing of seed. In these field and greenhouse tests (1952–1955), 8 separate experiments were made. They were run in quadruplicate; in the field each replicate consisted of a 25-ft. row with seed planted about 12 seeds per ft., and in the greenhouse each replicate consisted of 1 row in a flat (25 seeds per row). The treatments were of 2 types: seed dressing with chemicals and side dressing of seed with chemicals. During the course of these investigations, the liquid and dust treatments included formalin and CBP-55 (55 per cent chlorobromopropene) applied as side dressing, and sulfur, calcium sulfamate, Arasan (50 per cent bis(dimethylthiocarbamyol) disulfide), New Improved Ceresan (5 per cent ethylmercury phosphate), Terraclor (20 per cent pentachloronitrobenzene), Anticarie (40 per cent hexachlorobenzene), Acti-dione (3-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide), Ceresan M (7.7 per cent *N*-(ethylmercuri)-*p*-toluenesulfonanilide) applied in excess as seed treatments, except for Acti-dione which was employed at the concentration of 250 or 1000 ppm.

The treatments, with 1 exception, did not decrease infection from soil-borne inocula. Acti-dione decreased emergence at the concentrations used, but the emerged seedlings were free of visible infection. Proper concentrations of this compound might prove satisfactory. Formalin at a 20 per cent concentration was phytotoxic when applied as a side dressing at the rate of 1.5 ml per linear foot; at 5 per cent concentration and with the same rate of application, it caused a decrease in stand but had no apparent effect on infection. CBP-55 did not affect pathogenicity nor did it decrease emergence when applied at the rate of 0.1 ml per linear foot.

Variability of P. carthami and varietal reaction.—Variability of the pathogen was tested by inoculating several safflower varieties with various isolates. In 1950, 4 collections from 3 locations (Longmont, Colorado; Lincoln, Nebraska; and Scottsbluff, Nebraska) were tested on 13 varieties. Plants were inoculated when in the 6- to 8-leaf stage. No conclusive proof could be noted of the existence of strains of the pathogen. In 1951, 3 collections from Scottsbluff, Nebraska, tested on 6 varieties and 2 unnamed safflower selections, gave results similar to the 1950 data. In 1953, 2 different types of pustules on the

same leaf of the N-8 variety were selected as possible sources of different races, but when they were used as sources of inocula for 10 different varieties, definite differences could not be detected.

In these tests, varietal differences in rust reaction were noted. In general, N-1 appeared moderately resistant; small pustules and slight chlorosis developed. Varieties N-4, N-8, and N-9 had no yellowing around the pustules, but N-5 had a moderate amount of chlorosis around the pustule area although it was not similar to nor as extensive as the chlorosis exhibited by N-3 (Fig. 1). Cormack and Harper (3) state that N-3 possesses some resistance to *P. carthami* but did not elaborate on the symptoms.

Varietal reaction to the disease phase resulting from soil-borne inocula was indicated by tests with the varieties used for leaf infection. In 1952, 9 varieties showed differences in the percentage of rusted plants in field tests. In the variety N-1, only 1 per cent of the plants were infected in comparison to 27, 44, and 60 per cent, respectively, for the varieties N-3, N-6, and N-8. In the N-3 variety, most of the lesions were on the upper part of the hypocotyl; very few appeared on the lower part of the hypocotyl, as was true for the other varieties.

In greenhouse tests, the percentage of infected seedlings from the soil-borne inocula was 31, 54, and 62 per cent for N-1, N-3, and N-8 varieties, respectively. In the N-1 and N-3 varieties, only 2 per cent of the base of hypocotyls was infected compared to 22 per cent for N-8. Whether or not this has any bearing on resistance is a moot question, but this difference did exist in most cases where comparisons were made between the N-3 and N-8 varieties.

Possibly, resistance to infection by urediospores may be somewhat different from infection by teliospores. Also, entrance and establishment of infection by teliospores may not be as difficult as is the case with urediospores. This may explain differences in varietal reaction to leaf infection by urediospores as compared to seedling infection by teliospores. Sackston (5) in 1953 reported that the named varieties N-8, N-10, N-852, N-6, and Indian appeared very susceptible in field observations, a finding confirmed by our experimental results.

During the testing of varieties and selections for their reaction to rust, it was found in 1953 that 0.5 per cent of the seedlings of the resistant variety W.O. 14 were susceptible to infection by soil-borne inocula. This indicates either variability in the fungus or heterozygosity in the variety. The facts that *P. carthami* is an autoecious fungus and that infection from teliospore germ tubes is a common occurrence indicate that variability in the organism is not likely to occur. Tests on safflower varieties containing a wide range of germ plasm might establish the presence of races.

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COMPARATIVE STUDIES OF THE VIRUS DISEASES WESTERN X LITTLE CHERRY AND LITTLE CHERRY [1]

Jack M. Wilks[2] and J. A. Milbrath[3]

SUMMARY

Little cherry and western X little cherry have a similar range of symptoms in the commonly grown sweet cherry varieties on mazzard understock. The effect of each virus is limited to failure of the fruit to reach proper size and maturity. Both viruses cause a reduction in the weight and in the soluble solids content of fruit and a reduction in the weight and oil content of seed. A number of varieties and unnamed seedlings that produce a red leaf reaction when inoculated with little cherry virus produce similar symptoms when inoculated with western X little cherry virus; however, the 2 viruses cause distinctly different reactions on certain indicator hosts. Western X little cherry virus induces wilt and decline in sweet and sour cherry on mahaleb understock. In these hosts, little cherry virus induces fruit symptoms similar to those induced in cherry on mazzard understock but no foliage symptoms. Western X little cherry virus causes western X-disease in peach and a red-leaf condition in western chokecherry, *Prunus demissa* (Nutt.) Walp. These hosts are immune to little cherry virus. The spread of little cherry virus from tree to tree and within individual sweet cherry trees is much more rapid than the spread of western X little cherry virus. There appears to be ample justification for considering little cherry and western X little cherry to be distinct diseases.

The virus diseases of sweet cherry known as western X little cherry, buckskin, albino, little cherry, and small bitter cherry are treated as separate diseases in Agriculture Handbook No. 10[4]. Since the symptoms of these diseases are very similar in a number of hosts, the diseases are often referred to as belonging to the "little cherry group."

This paper compares 2 of these diseases: little cherry in the Kootenay region of British Columbia and western X little cherry as it occurs at The Dalles, Oregon.

METHODS AND MATERIALS.—The serious nature of these diseases and the danger of introducing one of them into a new area prevented a direct comparison in the same location. For little cherry studies, plots were established in the Creston and Kootenay Bay districts of British Columbia. The sources for all inoculations were trees of the Lambert variety of sweet cherry (*Prunus avium* L.) in the Kootenay Bay orchard. These trees had consistently produced severe symptoms. For western X little cherry, studies were conducted on the Mid-Columbia Branch Experiment Station at The Dalles, Oregon. A tree of the Bing variety of sweet cherry, coded as E10 in the Kronberg orchard at The Dalles, was used as source of inoculum. All inoculations were made by "T" budding or "chip" budding with buds from either 1-year-old wood or 1- to 3-year-old fruit spurs. For a few inoculations, whip grafts were used. Several criteria were employed in making the comparisons between these 2 diseases.

RESULTS. — *Red foliage indicator varieties.* — A number of sweet cherry varieties and seedlings produce a red leaf pattern in early autumn when they are inoculated with little cherry virus[5]. Five of these varieties and seedlings, S-5-16(7), S-7-10(SF), S-8-4(SF), Sam, and Star, which produce the strongest symptoms, were established on separate arms of mazzard (*P. avium* L.) seedlings at The Dalles, Oregon. Each variety or seedling was established on 4 different trees and inoculated in the spring of 1953 by chip budding from the E10 source tree of western X little cherry virus.

By fall, all 5 varieties showed red foliage symptoms similar to those characteristic of little cherry. The symptoms varied from a dark interveinal reddening in the Star variety to a less intense reddening that followed the veins and leaf margins in the variety Sam and in the seedlings S-5-16(7), S-7-10(SF), and S-8-4(SF). Little cherry virus induces a more intense interveinal reddening in these varieties and seedlings than does western X little cherry virus; otherwise, the reaction is similar.

The foliage of western chokecherry (*P. demissa* (Nutt.) Walp.) becomes a brilliant red when infected

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TABLE 1.—*Average trunk diameter and terminal growth of healthy Lambert sweet cherry trees and of comparable trees with little cherry virus*

Rootstock	Terminal growth 1952	Terminal growth 1953	Trunk diameter[a] 1953
	in.	in.	in.
Mahaleb			
Control trees	31-34[b]	17-25	2.35
Inoculated trees	22-28	8-15	1.86
Mazzard			
Control trees	29-46	15-28	2.13
Inoculated trees	25-34	11-16	1.71

[a] Measured at a point 1 ft. above the graft.
[b] Range of lengths recorded.

with western X little cherry virus (6). At Creston in 1951 and again in 1952, 5 western chokecherry trees were inoculated with the virus that causes little cherry. None of these trees has shown any tendency for early fall coloration, although all the 1952 buds made union and the buds grew on 2 of the 5 trees. Inoculations back to the indicator variety Star have failed to demonstrate passage of the virus through chokecherry.

Sweet and sour cherry on mazzard and mahaleb understocks.—The nature of the host reaction to several viruses in the little cherry group has been shown to depend on whether the cherry tree is established on mazzard or on mahaleb (*P. mahaleb* L.) understocks[6]. Tests of the understock effect on symptom expression of western X little cherry were already in progress or were completed, making investigation unnecessary during these studies. Since no previous studies on the influence of understock on the reaction to little cherry virus had been made, 20 trees of Lambert sweet cherry and 20 of Montmorency sour cherry (*P. cerasus* L.) were established in field plots. Ten trees of each variety were on mazzard, and 10 were on mahaleb understock. The trees were planted in the spring of 1951, and 5 trees of each variety on each understock were inoculated with 3–5 buds from a little cherry source in Kootenay Bay in the summer of 1951.

In 1952, the inoculated Lambert trees grew less vigorously than did the noninoculated trees (Table 1). On mahaleb understock, terminal growth of inoculated trees was 26.2 per cent less than that of the checks, and trunk diameter was 32.1 per cent less. On mazzard understock, the corresponding data were 18.3 and 27.6 per cent, respectively. The Lambert cherry trees on mahaleb understock showed slight upcurling and yellow-green coloring in the leaves. The wilt and decline symptoms that result from infection with the virus that causes western X little cherry were not evident in any of these trees, however, and they were still growing fairly normally at the end of the 1954 season. The Lambert trees bore fruit in 1953 and again in 1954. All fruits on the inoculated trees, whether on mahaleb or on mazzard understock, were small, dull red, angular, and pointed—symptoms typical of little cherry. Thus the understock had no effect on the expression of fruit symptoms.

The inoculated Montmorency trees displayed a severe ring spot shock reaction the first year after inoculation. These test trees had been propagated from virus-free sources, and the little cherry virus inoculum was contaminated with a ring spot virus. By 1953, the trees had recovered from this shock reaction and produced a small crop of fruits. Four of the 5 Montmorency trees on mahaleb understock produced small, round, light-colored fruits on branches adjacent to the points of inoculation. The trees on mazzard understock showed no fruit symptoms until 1954, when a similar fruit symptom appeared. There were no apparent differences in foliage or fruit symptoms in trees on the 2 understocks, and none of the trees developed symptoms of wilt and decline. Subsequent transmission to the Star variety of sweet cherry, with the development of red leaf symptoms, demonstrated that Montmorency trees showing these symptoms were infected with the little cherry virus.

Peach.—Western X little cherry is caused by the virus that causes western X-disease in peach[6]. No varietal resistance to this disease has been reported for peach, and symptoms have been produced on several varieties by budding with infected buds from cherry trees showing western X little cherry symptoms[6]. No further work on western X-disease was done, but comparable studies were made with little cherry virus. Seventeen peach trees, including the varieties Elberta, Hale, Rochester, and Vedette, were inoculated in 1951 by means of buds from trees known to be infected with little cherry virus. In the spring of 1952, the trees showed a shock reaction to ring spot virus contaminants that apparently prevented the survival of the sweet cherry buds. There was good recovery by the summer of 1952, and all the trees were reinoculated at that time by means of buds from the source trees that were used in the previous year's budding. These sweet cherry buds made good union and considerable sweet cherry growth occurred on 8 of the 17 trees. Over the 2-year period, a total of 198 infected sweet cherry buds was placed in the 17 peach trees. By the end of the 1954 growing season, none of these trees had developed symptoms that might be interpreted as those of western X-disease. No symptoms of little cherry developed when buds from these peach trees subsequently were budded into the Star variety of sweet cherry. This showed that the peach was not carrying the virus symptomlessly.

Weight and sugar content of the fruit.—Comparative studies were made of the weight and of the soluble-solids content of fruits infected with each of the 2 viruses. A Bausch and Lomb hand refractometer was used for tests of the soluble-solids content. Each fruit

[6] Reeves, E. L., E. C. Blodgett, T. B. Lott, J. A. Milbrath, B. L. Richards, and S. M. Zeller. 1951. Western X-disease. In Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handbook 10, p. 43-52.

TABLE 2.—*Average weight and soluble solids content of healthy fruit and of fruit showing symptoms*

Disease	Average weight[a]		Average soluble solids[b]	
	Healthy	Diseased	Healthy	Diseased
	g	g	%	%
Western X little cherry	8.31	3.6	21.9	9.7
Little cherry	7.43	3.94	18.8	10.6

[a] Based on 150–200 fruits.
[b] Based on 15–20 fruits.

TABLE 3.—*Average weight and oil content of seeds from healthy fruit and from fruit showing symptoms*

Disease	Average weight		Average oil content	
	Healthy	Diseased	Healthy	Diseased
	g	g	g	g
Western X little cherry[a]	0.061	0.024	0.022	0.002
Little cherry	0.065	0.045	0.019	0.011

[a] Jones, L., J. M. Wilks, and H. E. Williams. Unpublished data.

was tested by making a cut along the suture line, squeezing a small amount of juice on the refractometer, and taking the reading of each fruit individually.

Fruits from trees infected with western X little cherry virus weighed 56.7 per cent less than those from the controls. Fruits from trees infected with little cherry virus weighed 47.0 per cent less than those from the control trees (Table 2). The soluble-solids content of symptomatic fruit from trees inoculated with western X little cherry virus was 55.7 per cent less than that of fruit from noninoculated trees (Table 2). That of fruit from trees inoculated with little cherry virus was 43.6 per cent less than that of the controls.

Effect on weight and oil content of the seed.—Studies were made of the weight and oil content of seeds from healthy trees and of seeds from trees infected with little cherry virus. The varieties were Bing, Lambert, and Royal Ann. Twenty-five air-dried seeds were used in the weight determinations; 50 were used in each oil determination. For each test, 50 seeds were ground, placed in a Soxhlet extractor, and extracted in 125 ml of ether for 2 periods of 24 hours each. After the first extraction, the preparation was ground into a finer powder and the extraction procedure repeated. At the end of the second extraction, the ether was evaporated and the weight of the oil that remained was determined. Since similar experiments[7] had already been conducted with seeds from trees infected with western X little cherry virus, this work was not repeated.

At the time of collection, no differences in appearance could be detected between the seeds from healthy trees and those from diseased trees. When air-dried, however, seeds from diseased trees shrivelled and turned brown whereas those from healthy trees did not. In this test, Western X little cherry virus caused 60.7 per cent reduction in the weight of the seed, whereas little cherry virus caused 31.8 per cent reduction (Table 3).

The oil extraction methods differed for the 2 diseases, so the total oil content for seed from trees infected with each disease cannot be compared directly; however, the oil content of diseased seed taken as a percentage of the oil content of the healthy seed provides a basis for comparison of the 2 diseases. Western X little cherry virus caused a 90.0 per cent reduction compared with a 42.1 per cent reduction for little cherry virus.

Rate of virus movement in sweet cherry.—Surveys of the spread of little cherry virus conducted over a 2-year period in Creston Valley showed an increase in number of infected trees from 155 to 723 or an increase of 367 per cent[8]. The movement of western X little cherry virus in orchards has never been this rapid. Western X little cherry surveys conducted in Oregon over the past 5 years showed an increase in number of infected trees from 102 to 171, an increase of only 67.6 per cent for the 4-year period.

The movement in individual trees was studied by making bud inoculations and recording the subsequent spread annually. Records were taken over a period of 2 years in 12 trees infected with western X little cherry virus. Rate of movement of the virus varied from tree to tree. In some trees, only spurs adjacent to the point of inoculation showed symptoms after 2 years. In others, scattered spurs 4–6 ft. from the point of inoculation were showing symptoms. The average annual movement of the virus upward from the point of inoculation was about 15–20 in.

The results of records taken on the movement of little cherry virus in 3-year-old trees of the Star variety showed that the virus moved 35–38 in. into the tips of inoculated branches the first year after inoculation. The following year, the virus had spread throughout the remaining healthy branches in the tree. For each of the diseases, these results agreed with field observations of spread in larger numbers of trees.

DISCUSSION.—The differences that have been demonstrated in this study indicate that western X little cherry and little cherry should be considered as 2 distinct diseases. This might be interpreted as an indication either of the existence of 2 distinct viruses or of strains of the same virus. There appears to be more justification for accepting the latter viewpoint.

Justification for considering them separate diseases was provided by their varying host reactions in sweet and sour cherry on mahaleb understock, by their different behavior on peach and chokecherry, and by

[7] Jones, L., J. M. Wilks, and H. E. Williams. Unpublished data.

[8] Foster, W. R., and I. C. MacSwan. 1949. Report of the provincial plant pathologist. Brit. Columbia Dept. Agr. 44th Ann. Rpt. 1949. W83-W88.

the distinct difference in the rate of movement of the causal virus through mature cherry trees, as detected by symptom expression.

That the 2 diseases are caused by strains of the same virus was suggested by the similar reactions obtained on the red foliage indicator varieties; by their similar effects on the size, shape, and soluble-solids content of the fruit; and by the comparable effects of the 2 viruses on the weights and oil contents of the seed.

Until more reliable techniques for identification of stone fruit viruses are developed, little cherry and western X little cherry should be considered as 2 distinct diseases that may be caused by different strains of the same virus.

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ELIMINATION OF ASPERMY VIRUS FROM THE NIGHTINGALE CHRYSANTHEMUM[1]

Francis O. Holmes

SUMMARY

The Nightingale chrysanthemum was available formerly only as a doubly infected carrier of tomato-aspermy virus and a mosaic-type virus. Now it has been freed from the former contaminant by propagation of scions cut from the extreme tips of branches. The scions, 4 to 8 mm in length, were grafted onto healthy plants of another variety, the Good News chrysanthemum. Those that survived and developed new foliage were tested for the presence of aspermy virus by mechanical inoculations to young Turkish tobacco plants. Among 244 scions tested, 231 still carried aspermy virus, but 13 did not although they still harbored the mosaic-type virus. Clones of the Nightingale chrysanthemum, improved in the sense that they were free from 1 of the 2 original contaminants, were produced by the rooting of cuttings taken from 5 representatives of the 13 aspermy-virus-free scions.

Because of vegetative reproduction, the florist's chrysanthemum (*Chrysanthemum morifolium* Ram.) is subjected continuously to the danger of repeated viral infection. The chrysanthemum variety Nightingale has presented an especially interesting problem to plant pathologists. In recent years, no healthy stock of this variety has been available. Infected stocks have constituted reservoirs of disease endangering other susceptible plants.

The clone of the Nightingale chrysanthemum used in the present investigation[2] carried 2 viruses, although it appeared healthy and grew vigorously. The first was that of tomato aspermy,[3] which is usually detected by its mechanical transmissibility to young plants of tobacco (*Nicotiana tabacum* L.). The second was that of a mosaic, resembling Noordam's B mosaic (5),[3] the presence of which can be demonstrated by grafting diseased scions onto the highly susceptible chrysanthemum variety Good News (2).[2]

Aspermy virus is much more undesirable in a chrysanthemum variety than is the mosaic-type virus, because of the potential hazard of its transmission to other crop plants (3). The tomato, *Lycopersicon esculentum* Mill., bears almost no useful fruit after it is infected (1); indeed, it forms very few functional pollen cells or ovules in the presence of aspermy disease (4). Distribution of symptomless but infected chrysanthemum varieties would tend to bring aspermy virus into localities where potential aphid vectors occur. On this account, attention was focused on an attempt to eliminate aspermy disease from the infected Nightingale chrysanthemum.

A substantial number of host plants, or parts of host plants, can be freed from specific viruses by special techniques. Among methods thus far developed, the most generally applicable are heat treatments and propagation of uninvaded parts of plants by rooting of cuttings or by establishment of small scions.

Tomato-aspermy virus produces blotchy chlorotic-mottling patterns in many of its experimental hosts. This phenomenon indicates erratic distribution of the disease agent in infected plants and would suggest that improved stocks might be obtained by the scion-grafting technique. Furthermore, the failure of the virus to produce appreciable disease in the Nightingale chrysanthemum suggests that it enters growing tissues of this plant only after they are mature enough to be but little affected by its presence. The exact distribution of the virus in the chrysanthemum plant is difficult to demonstrate, because the induced disease is masked and subinoculations from diseased leaves are

[1] Accepted for publication June 28, 1956.

[2] Cuttings of the Nightingale chrysanthemum were made available by Dr. Philip Brierley, U. S. Dept. of Agriculture, Beltsville, Maryland; cuttings of the Good News chrysanthemum by Dr. C. F. Olsen, Ashtabula, Ohio.

[3] Tomato-aspermy virus has been regarded by some investigators as a strain of cucumber-mosaic virus (*Marmor cucumeris* H.); this supposed relationship is still controversial. The possible relationship of chrysanthemum-B-mosaic virus to other viruses has not been established.

not invariably successful.

Small wedge-shaped scions cut from the extreme tips of vegetative branches of the Nightingale chrysanthemum were introduced into clefts cut in the truncated tops of stems of the Good News chrysanthemum. The Good News understocks were known to be free of the 2 viruses that were recognized as present in the Nightingale variety. Introduced tips were bound firmly in place by the use of tacky crepe-rubber strips. The scions first used were about 8 mm in length. Progressively smaller tips of branches were used until the scions in about half of the attempted grafts failed to survive. Some of the later scions were only about 4 mm in length.

Some scions grew promptly and were ready for testing within a few weeks. Others grew very slowly. When each surviving scion developed foliage, its maturing leaves were tested for the presence or absence of aspermy virus. The leaf-edge method of Yarwood (6), with No. 320 Carborundum powder, was used in this subinoculation to young plants of Turkish tobacco. Scions that proved to contain aspermy virus were discarded at once, whether or not they had shown evidence of containing the mosaic virus. Even when leaves were taken from diseased plants, however, not every attempt to isolate aspermy virus from the Nightingale chrysanthemum proved successful. This was not thought to mean that the tested leaves from diseased plants lacked infectivity but that the test for it was of such low efficiency as to be indecisive when negative. Scions that failed to yield the virus in a short series of preliminary inoculations were tested again in repeated comparisons with simultaneous inoculations from the original infected stock plants.

The first scion to give evidence that aspermy virus had been eliminated (scion number 2813 H) was tested as follows. The apex of the growing scion was removed and grafted into an additional Good News understock. Later, the apices of several side branches that developed from lower portions of the original scion were propagated similarly on other Good News plants. When each new scion had grown to a length of about 3 in., the second-scion-generation plants were tested by subinoculations to young Turkish tobacco plants, in comparison with simultaneous subinoculations from the original Nightingale line. Among 33 young tobacco plants inoculated from this clone, none became infected. Among 33 comparable tobacco plants simultaneously inoculated from the original Nightingale chrysanthemum clone, 17 became infected. Thus in 17 pairs of effective tests, only the original Nightingale stock and never the new plants derived from scion 2813 H showed evidence of carrying aspermy virus.

Among a total of 426 small branch-tip scions that were cut from Nightingale chrysanthemum plants and introduced into Good News chrysanthemum understocks, 182 died before they could be tested. Eventually 231 of the survivors proved to contain aspermy virus; these were discarded. Only the scion described above and 12 additional scions consistently failed to yield this virus. In all tests in which the corresponding diseased control plants proved effective sources of aspermy virus, each of the 12 scions failed to yield the virus; there were 7 such tests on 5 scions, 8 on 5, and 9 on 2. In addition, these 12 scions failed in a total of 77 cases to yield virus in tests in which the known diseased plants also failed. In no test did they themselves yield virus.

The scion branches from which aspermy virus seemed to have been eliminated were allowed to grow until they were about 1 ft. in length. Then they were used to provide 4- to 6-in. cuttings that subsequently were rooted and established on their own roots as separate plants. The rooted cuttings grew vigorously and were regarded as constituting aspermy-virus-free clones of the Nightingale chrysanthemum. Altogether, 5 of the selected scions provided cuttings that were thus established as separate plants free from aspermy virus.

All of the 13 scions from which no aspermy virus could be obtained showed the presence of the mosaic virus. This may mean that the latter virus is more difficult to eliminate than is the former, or it may merely mean that coincidence of exclusion did not occur. Further studies will be necessary to determine whether the mosaic virus can be eliminated by a grafting technique. During the course of the experiments reported here, attempts were made to eliminate both viruses, or either of them, by other means such as heat or the application of chemotherapeutic measures. Only the mechanical technique has given evidence of success thus far. The simultaneous presence of 2 agents of disease in the original clone of Nightingale chrysanthemum, however, still offers a good opportunity to use this stock of plants in appraisal of other experimental procedures.

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STUDY OF THE VALUE OF PINEAPPLE PLANTS AS SOURCES OF THE MEALYBUG WILT FACTOR[1]

Walter Carter and K. Ito

SUMMARY

The value of pineapple plants as sources of the mealybug wilt factor was tested over a period of 2 growth cycles. One hundred families from 6 clones were distributed in 6 different locations on the Island of Oahu. There were 6 tests made in the first cycle and 7 in the second.

Most of the positive sources occurred in the resistant clones; only in a few instances were positive sources found in susceptible clones.

Only 1 positive source plant was positive in all tests in the first cycle and none tested positive in more than 4 out of 7 tests in the second cycle in spite of the use of large numbers of insects for each test transfer. The data indicate that the mealybug wilt factor is a fluctuating latent factor limited to certain plants and modified only slightly by local environmental and agronomic conditions.

The nature of the disease of pineapple plants (*Ananas comosus* (L.) Merr.) that follows the feeding of the pineapple mealybug (*Pseudococcus brevipes* (Ckl.)) has been the subject of study for many years.

Carter (2) reviewed the progress of this study in 1939 and reiterated the previous conclusion that the disease is due to the injection into the plant of a toxic substance developed by the mealybug on the plant previously fed upon. This rather simple explanation proved adequate as the basis for the development of control measures that still depend, not on the elimination of established mealybug populations, but on their reduction in size.

With continued study, certain data accrued that appeared at variance with the toxin hypothesis. These were brought together by Carter in 1951 (3), and the whole subject again reviewed in 1952. This last review (4), which appears to have escaped the attention of more recent reviewers (1, 7), makes it clear that the etiology of mealybug wilt does not fit that of any known virus disease nor can it be ascribed to a simple pineapple-to-pineapple feeding sequence of the mealybug. The suggestion was made, however, that a latent virus precursor may be involved.

One of the principal difficulties in experimental work with mealybug wilt is that of reproducing results. It was supposed, after it was learned that pineapple plants differed in their value as sources for the mealybug wilt factor, that this difficulty would be greatly reduced by using plants of known positive or negative source value in experiments. It was shown by Carter (3), however, that even colonies grown on the same leaf of a positive source plant may,or may not, induce wilt in test plants.

In that same paper are data indicating that gross field collections of mealybugs from fields in different localities differed in their ability to induce wilt. The present study was begun as a consequence of that datum, with the sole object of determining what degree of stability in source value could be demonstrated when several clones, known to differ in their source value, were planted in several locations and tested throughout the growth cycle of the crop.

MATERIALS AND METHODS.—Mother plants were selected from 6 clones of the commercial pineapple variety Smooth Cayenne that had been grown by vegetative reproduction for many years. They were known to differ in their susceptibility to mealybug wilt and also in their source value, i.e. whether or not they are positive sources of the wilt factor. Positive source plants are defined as those that confer on mealybugs feeding on them the ability to induce wilt in test plants to which the insects are transferred. Feeding on negative source plants does not confer this ability (4). All the mother plants selected for these tests were themselves entirely symptom-free. Two of the clones used were resistant to mealybug wilt and known from previous studies to be positive source plants. Three were susceptible clones, and 1 was randomly selected field-run material without family relationship. Where possible, plants were selected that had 6 slips, so that sisters of the same family could be tested at each of 6 locations on the Island of Oahu; actually there were few of these and many

TABLE 1.—*Number of positive source plants (of the wilt factor) among the original family mother plants of pineapple*

Clone	Mother plants tested	
	No.	Positive[a]
FR	28	2
LH 8	34	3
LH 20	27	0
L 69	30	0
573	23	18
666	13	12

[a] A mother plant was rated as a positive source if 100 mealybugs fed upon a slip from the plant induced wilt in the test plant subsequently fed upon.

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TABLE 2.—*Recovery of the wilt factor from sister slips of positive-source families in first growth cycles of field-run LH 8, LH 20, and L 69 pineapple clones planted in different locations and tested 6 times*

Mother plant	Incidence of wilt[a] among test plants that were fed upon by mealybug colonies that had fed previously on slips taken from indicated mother plants and planted in the location indicated					
	1	2	3	4	5	6
Field run						
1	–00000	–00–00	–00000	–00X00		–00000
2	000000	X00000	000000	000000	000000	000–00
3	X00000	000–00			000000	–00000
4			–00000	–00000		–000X0
5	–0X0X0	–00000			–00000	
LH 8						
1	X00000	000000			000000	
2			–00000	–00000		–00–0X
3	000000	000000			000000	X000X0
4	–00000	–00000			–00000	–X0–00
5			–00000	–00000		–0000X
LH 20						
1			–000X0	–0000X		–00000
2	000000	0000X0	000000	000000	000000	000–00
3			–00000	–00000		–0X000
4			000000	000000	0000X0	0X0000
5	000000	000000			X00000	
6	000000	000000	000000	00X000		000000
L 69						
1	000000	0X0000			000000	
2			–000X0	–00000		–00–00
3	–00000	–00000	–00000	–00000	–000X0	–00000
4	000000	000X0X	000000	000000	000000	
5	–00000	–000X0	–00000	–00000		–00000
6	–00000	–X0–00			–000XX	
7			–00000	–0000X		–00–00
8	–00000	–00XXX			–00X0X	
9	–00X0–	–00000	–00000	–00000	–00000	–00000

[a] The 6 adjacent figures in any 1 group are the results of 6 tests made at intervals during the growing cycle. A dash indicates no test, X that wilt was produced, and 0 that no wilt was produced.

additional families had to be included to make up the 100-plant lots for each location.

When the selection was made, 1 leaf was pulled from 1 slip from each mother plant and tested for its source value by caging 100 mealybugs on it for 10 days and then transferring the colony to a test plant. The colony was allowed to feed on the test plant for 5 days.

Although there were no data to show that all the slips from a single mother plant would react similarly as a source plant, the initial testing of 1 slip was at least a representative sampling of the source status of plants at the beginning of the experiment.

Further tests began when the plantings in the 6 locations were approximately 7 months old. A single leaf was pulled from each plant, and 5 gravid female mealybugs were caged thereon. After 2 weeks, the colony of first-instar mealybugs derived from these females was transferred to a test plant growing in the field under standard plantation practice. After 10 days, these colonies were killed by spraying with an insecticide. It should be pointed out that the colonies produced in 2 weeks by 5 gravid females are usually very large, comprising up to 2 or 3 hundred crawlers and first-instar larvae.

This method was followed until the plants fruited for the first or plant crop, then leaves from the fruit crowns were used as source material. With the development of the ratoon suckers, leaves from these were used; finally, the crowns from the second or ratoon fruit were used. In all, 6 separate tests were

TABLE 3.—*Percentage of wilt in test plants fed upon by mealybug colonies previously fed on pineapple clones 573 and 666 from 6 locations in 6 tests during the first cycle.*

Clone and location	Test						Average
	1	2	3	4	5	6	
Clone 573							
1	75	57	93	29	36	23	52.2
2	63	43	29	40	57	21	42.2
3	50	43	43	38	50	57	46.8
4	38	50	36	38	21	46	38.2
5	63	50	36	43	43	38	45.5
6	25	36	57	44	79	0	40.2
Average	52.3	46.5	49.0	38.7	47.7	30.8	
Clone 666							
1	83	100	33	33	17	33	49.8
2	50	50	50	33	50	0	38.8
3	83	50	67	17	50	50	52.8
4	67	50	50	50	33	100	58.3
5	17	17	50	60	33	33	35.0
6	50	0	33	50	50	0	30.5
Average	58.3	44.5	47.2	40.5	38.8	36.0	

TABLE 4.—*Recovery of wilt factor from sister slips of pineapple clone 666 planted in 6 locations and tested 6 times in first cycle*

Mother plant	Incidence of wilt[a] among test plants that were fed upon by mealybug colonies that had fed previously on slips taken from indicated mother plants and planted in the location indicated					
	1	2	3	4	5	6
1			00X000	000X0X		0000X0
2	XX000X	XXX0X0			00X000	
3	XX0X00	X000X0	X0X00X	00XXXX		X00X00
4	XXXX00	000000			00X000	
					0X0X0X	
5			X00XX0		X00000	
6	XX0000			XX000–		
7		0XXX00			00X–00	
8			XXX00X			X0XXX0
9	XX0000			XX000X		
10		XX000X			000XXX	
11			XXX0X0	X0X00X		00XXX0
12	0XX0XX	00XX00			X000XX	
13			XX00XX	XXXXXX		000000

[a] The 6 adjacent figures in any 1 group are the results of 6 tests made at intervals during the growing cycle. A dash indicates no test, X that wilt was produced and 0 that no wilt was produced.

completed between 1949 and 1952 in the first cycle of 2 crops.[2]

Progeny of the plant crop were used to plant a second cycle, but because of the small number of slips that developed, the planting was limited to 3 locations and to 60 plants per location. Seven tests were completed between 1951 and 1953. The locations used were essentially those from which the previous data were obtained (3) and differed in rainfall, sunlight, and soil fertility. The vegetative vigor of the plants grown in these locations was conditioned by these environmental factors and also by the agronomic practices employed by the several plantations represented.

RESULTS.—In the original test of the single slips from each of 155 of the mother plants from which came the planting pieces (slips) used in the experiment, 35 slips were positive (Table 1); of these, 30 were from the resistant clones 573 and 666, 3 were from a susceptible clone, LH 8, and 2 were from the field run material.

In the subsequent first-cycle tests, positive sources were so few in all except the 2 resistant clones that little indication of an effect of locality was obtained. The record for the 4 clones (including field-run) that were almost completely negative as sources in both cycles is shown in Table 2. Only those families (the progeny of 1 mother plant) that were positive sources at some time during the experiment are included. There were 6 positive cases in clone LH 8, and 5 of these were from the same locality. L 69 had 16 positive tests, of which 8 were from one locality and 5 from another.

Results with the 2 resistant clones 573 and 666 are summarized in Tables 3 and 4. The averages of all first-cycle tests show no important differences among locations, but there are real differences among locations in any one test. For example, in Test 2, all tests with clone 666 from Location 1 were positive, whereas all were negative from Location 6. There are also significant differences among the several tests from a single location.

Table 4 shows the source value of clone 666 sister slips by location and test. Two of these plants were negative throughout all 6 tests. One, a sister of an entirely negative plant, was positive in all 6 tests. All the other plants were positive from 1 to 4 times in the 6 tests.

[2] A pineapple "cycle" comprises the first, or plant crop, which is derived from the planting of a vegetative offshoot, and the ratoon crop, which arises as a sucker from the stem of the plant crop plant. Theoretically an unlimited number of ratoons could develop in succession as suckers from the preceding crop plants, but in Hawaii, with few exceptions, only a plant crop and first ratoon are grown before the field is ploughed under and replanted.

TABLE 5.—*Recovery of wilt factor from plants of pineapple clone 666 in 7 tests made in the second cycle in 3 locations*[a]

Mother plant[b]	Location A	Location B	Location C
K95	0000000		
W95	0X0000X	000X000	X000–00
K96		X000X0X	
K97	X0X000X	X0000X0	X00X0X0
			X0XX–00
W97	0X0000X		0000000
B97	X0X000X	X0XX0X0	
K98	X0XX–0		
K99	X00X–XX	X00X0XX	00X00X0
		000X0X0	
B99	X00000X	X0XX000	X00X000
W99			X00X000
R99		X0XX–0X	X000000
K100	X000X–0	00X0–XX	00X00X0

[a] The 7 adjacent figures in any 1 group are the results of 7 tests made at intervals during the growing cycle. A dash indicates no test, X that wilt was produced, and 0 that no wilt was produced.

[b] All were positive sources in at least 1 test in the first cycle.

TABLE 6.—*Number of positive sources of the wilt factor found among second-cycle pineapple slips taken from plants classified as positive or negative sources during the first cycle*

Clone	Source plant status in first cycle	Second-cycle source plants	
		Tested	Positive
FR	+	1	0
	−	26	0
LH 8	+	4	0
	−	15	2
LH 20	+	10	3
	−	24	1
L 69	+	1	0
	−	18	2
573	+	48	43
	−	6	2
666	+	27	25
	−	0	0

Results with clone 666 from the second cycle also were variable (Table 5). All these plants were derived from first-cycle plants that tested as positive in at least 1 test in the first cycle. Despite this, 1 plant tested negative in all 7 tests and none tested positive in more than 4 of 7 tests.

Table 6 summarizes results from the plant families that were included in both cycle tests. The status of the field-run plants remained the same in both cycles; clones LH 8, LH 20, and L 69 showed a few plants completely negative in the first cycle and positive in the second. Clone LH 20 showed the reverse trend: 7 of 10 plants positive in the first cycle were completely negative in the second.

Table 7 is a complete summary of the results for both cycles. From this table, the superior position of the 2 resistant clones as sources of the mealybug wilt factor is clearly shown. The measure of the instability of the plants in this respect is found in a comparison of the data for individual plants with those from individual tests. Any plant testing positive at any time during the cycle was included in the former, but when all the tests were considered the number positive was reduced by more than 50 per cent.

DISCUSSION.—It is clear that the predominating factor in determining whether a plant is or is not a positive source for the mealybug wilt factor is primarily a function of the plant itself; however, rather extreme differences in local environment and in agronomic practices may influence the expression of this characteristic. This is evident in the record of the 2 strongly positive clones 573 and 666, where extreme variation in the percentage of wilt occurred in any one test when the plants used as sources were grown in 6 locations.

Equally clear is the factor of variability in the performance of individual plants. Among the most positive source plants (clone 666), only 1 of 36 slips from 13 positive mother plants produced a plant that tested positive in all tests in the first cycle. Also variable is the response of sister slips from a single parent grown in different locations.

Differences in source value, subject to the extreme variation reported in this paper, appear to be clonal —at least, it is certain that some clones contain a much higher percentage of positive source plants and fluctuate less in this regard than do other clones. The Cayenne clones used were originally selected on the basis of vigor and other desirable characteristics and each clone sprang from a single mother plant.

These clonal differences in source value and the fluctuations observed during the growth cycle and from cycle to cycle might be accounted for by differences in concentration of some substratum on which the mealybugs developed their toxic secretions.

The maintenance and concentration of such a substratum could be influenced by gene-controlled factors (5, 6) that would account for clonal differences, and it could be subject to seasonal and environmental influences that might account for fluctuations during a cycle or from cycle to cycle.

There is as yet no satisfactory explanation for the

TABLE 7.—*Summarized data on the percentage of plants that were positive sources of the wilt factor during the first or second growth cycles of pineapple slips grown in all locations*

Cycle and clone	Families		Individual plants		Individual tests	
	No. tested	Percentage with 1 or more positive progeny	No. tested	Percentage positive	No.	Percentage positive
First						
FR	29	17	126	4	675	0.9
LH 8	35	14	120	4	627	1.0
LH 20	27	22	108	7	579	1.4
L 69	30	30	126	9	669	2.4
573	23	96	84	90	453	43.7
666	13	100	36	94	214	43.5
Second						
FR	11	0	27	0	180	0
LH 8	10	20	19	11	129	1.6
LH 20	12	25	34	12	227	3.5
L 69	7	29	19	11	127	2.4
573	23	87	54	83	364	34.9
666	13	100	27	93	181	35.9

initiation of the condition that makes a plant a symptomless positive source or for the current lack of it in some of the clones studied, nor has this study revealed any technique whereby fluctuations can be avoided and a consistently positive source made available.

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PRODUCTION OF PECTINASE, CELLULASES, AND GROWTH-PROMOTING SUBSTANCE BY CERATOSTOMELLA ULMI[1]

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SUMMARY

Culture fluids of several isolates of *Ceratostomella ulmi* (Schwartz) Buisman were tested for the presence of pectic and cellulolytic enzymes as well as for growth-regulator activity. Pectin depolymerase and cellulases of the C_1 and C_x types were found. No pectin methylesterase or polygalacturonase was found. Elongation of pea epicotyl sections, comparable to that induced with 5 ppm indoleacetic acid, was induced by diluted culture fluids.

Rapid onset of pathogenic wilt symptoms is often accompanied by or preceded by reduction in water-carrying capacity of xylem tissue (2, 6, 14). In general, however, sparsity of mycelial growth in the affected vessels appears to preclude direct plugging action only (18). Fungal metabolites of high activity, i.e. enzymes, and growth regulators might upset the normal host physiology and induce occlusion by gums and tyloses.

Facultative parasites such as certain wilt pathogens probably would be strong producers of exoenzymes, since they must be able to degrade organic material in order to subsist as saprophytes. These same enzymes may function in pathogenesis. Evidence that pectolytic enzymes may induce occluding gummosis in these wilts has been reported (8, 13, 19). In addition, the occurrence of tylosis is common (15, 20). This tylosis might result from an increase in plasticity of the walls of ray parenchyma cells by fungal enzyme action, or both gummosis and tylosis might result from an abnormal metabolism of host cells induced by fungal growth-regulators. Auxin production by fungi has been reported (5, 16).

Establishment of the pectinase complex as a factor in a tree wilt would broaden and strengthen the enzyme hypothesis as a general mechanism of gummosis and wilt. It is questionable, however, whether pectinases alone could account for tylosis.

For these reasons the production of certain enzymes and growth regulating factors by *Ceratostomella ulmi* (Schwartz) Buisman on selected substrates was investigated.

MATERIALS AND METHODS.—Screw-capped 4-oz. prescription bottles were used for all "still" cultures. These were placed in a horizontal position during incubation to provide maximum gaseous exchange. Cotton-stoppered 250-ml Erlenmeyer flasks were used for aerated cultures. Aeration was accomplished by means of a reciprocating shaker having fifty 4-in. strokes per minute.

Liquid media were used throughout. Those for pectinase studies contained as basal ingredients 2 g asparagine, 1 g yeast extract, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 5 g diammonium citrate, and 0.01 g $FeCl_3$ per liter. Citrate was omitted from media employed in cellulose studies. Carbon substrates, which differed with the individual experiments, are indicated in the section on results. The medium used for growth substance production contained 10 g dextrose, 5 g tryp-

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tone, 1 g asparagine, 1 g $MgSO_4 \cdot 7H_2O$, 5 g KH_2PO_4, and 0.01 g $FeCl_3$ per liter. The initial pH readings for these media were pH 5.3 ± 0.1, adjusted when necessary with HCl or NaOH. Twenty-ml aliquots were dispensed unless otherwise indicated. These media were autoclaved for 20 minutes at 15 lb. pressure.

A total of 7 pathogenic isolates of *C. ulmi* were tested. These were RI-A, RI-B RI-C, NJ6, NJ10, NJ "Black"; and NH 2.[2] Media were seeded with 1-ml portions of turbid conidial suspensions from 2- to 4-week-old potato-dextrose agar (PDA) slant subcultures. Cultures were incubated in darkness at 23 ± 1° C for stated periods.

Procedures for enzyme assay were similar to those of the following: pectin methyl esterase (titration)—Kertesz (10); polygalacturonase or depolymerase (viscosity)—Bell et al (3); polygalacturonase or deploymerase (paper chromatography)—Gothoskar et al (8); cellulase (fiber swelling)—Marsh (12); and cellulase Cx (viscosity)—Levinson and Reese (11).

In these enzyme assays, culture fluids were removed from flasks by means of sterile pipettes. Mycelium was avoided by tilting the flask and withdrawing the solution slowly from under or above the mycelial mat. Generally, no attempt was made to remove all spores, since freshly seeded media had no measurable effect upon the substrates within the assay periods used. Five-ml portions of these solutions were added to 10-ml portions of sterile substrate solutions. Toluene (0.1 per cent) was added to inhibit growth.

Viscometric assay was used to measure hydrolytic enzyme activities, since it is extremely sensitive to partial degradation of large polymers. The production of such large fragments, which might be liberated into the transpiration stream of the host, seemed to be of more significance in the pathogenic mechanism than more complete degradation, from the standpoint of both plugging and toxic effects (9, 14). Substrate solutions were buffered with 0.5 M citrate buffer (sodium citrate plus citric acid) at pH 4.2 for pectin and at pH 4.5 for methyl cellulose hydrolysis. Incubation and viscosity measurements were at 23 ± 1° C.

Cellulase activity of the C_1 type was detected by measuring any increase in weight of alkali-treated cotton fiber[3] resulting from exposure to culture fluids. These fluids were obtained by growing the fungus on 250 mg cotton fiber immersed in 20 ml of basal medium. After suitable incubation periods, the cotton was removed and placed in a glass funnel. The culture fluids were then filtered through this pad to remove mycelial fragments without the danger of adsorbing enzymes on other filtering materials. Fluid was squeezed from this pad by a rolling action with a glass rod. Fifteen ml of this filtrate was added to 250 mg of fresh cotton and incubated for 4 hours at 23 ± 1° C. Fiber swelling was then measured according to the Marsh procedure.

The production of growth factor in culture was detected by means of a pea-epicotyl-section test. Culture solutions were adjusted to pH 6.0. Ten-ml aliquots of whole or diluted culture fluid or check medium were poured into sterile petri plates. Dilutions were made with a 1 per cent sucrose[4] solution. Ten epicotyl sections of Alaska pea seedlings, which had been grown in darkness at 23 ± 1° C for 7 to 8 days, were prepared in the manner described by Galston and Hand (7) and placed into each solution. Growth of these sections was measured under a stereomicroscope at 20 magnifications after 18–20 hours of incubation at 23 ± 1° C.

RESULTS. — *Pectinase production.* — Production of pectin esterase (PE) was investigated by the alkali titration method. Three replicate cultures of each isolate were tested after each of several incubation periods. This experiment was repeated 3 times with the NJ "Black" isolate and once with the NJ10, NJ6, NH2, and RI-B isolates. Commercial PE[5] served as a check. A strong test for PE (3.8 ml 0.02 N NaOH in 20 hours at 23° C) was obtained with a 0.02 per cent solution of the commercial enzyme. No activity was obtained with isolates of *C. ulmi* during the 24-day culture period.

Hydrolysis of citrus pectin[6] following the addition of fungus filtrate was determined viscometrically. Filtrates were aliquots of those used for PE assay; therefore, replication was identical in the 2 tests. A marked increase in fluidity of pectin solutions treated with culture filtrates was found. Since results were comparable with all isolates, combined averages of these data are given (Fig. 1). Each point represents the average of 21 measurements. In these experiments, increased fluidity was compared with oven-dry weight yield of fungus mycelium. No activity was detected even after 20 hours' incubation of pectin with freshly seeded, unfiltered medium. After 3 days' growth, however, the average fluidity increase was 80 per cent, whereas dry-weight yield was only 6 per cent of the maximum attained (12–18 days). Culture fluids that were sterilized by filtration (Seitz) showed no reduction in activity, whereas heat sterilization completely destroyed activity. These results indicate that germinating spores as well as young mycelia actively produce pectolytic enzyme.

Since 2 hydrolytic enzymes of pectin are now known, experiments were conducted to determine which was present. Paper chromotography was used to detect monogalacturonic acid which may have resulted from this hydrolysis. Fluids from 5- and 7-day-old cultures of the various isolates were sterilized by filtration (Seitz), and 5-ml portions of these were added to sterile 1.5 per cent pectin or pectic acid solu-

[2] Isolates with numerical designations were kindly supplied by Dr. Roger U. Swingle, Ornamental Plants Section, U. S. Department of Agriculture, P. O. Box 70, Worthington, Ohio.

[3] Brunswick bleached cotton, Johnson and Johnson Company.

[4] Mallinckrodt Analytical Reagent Grade.

[5] Rohm and Haas Company, Pectin Esterase No. 5.

[6] Sunkist Growers Company, Pectin N.F.

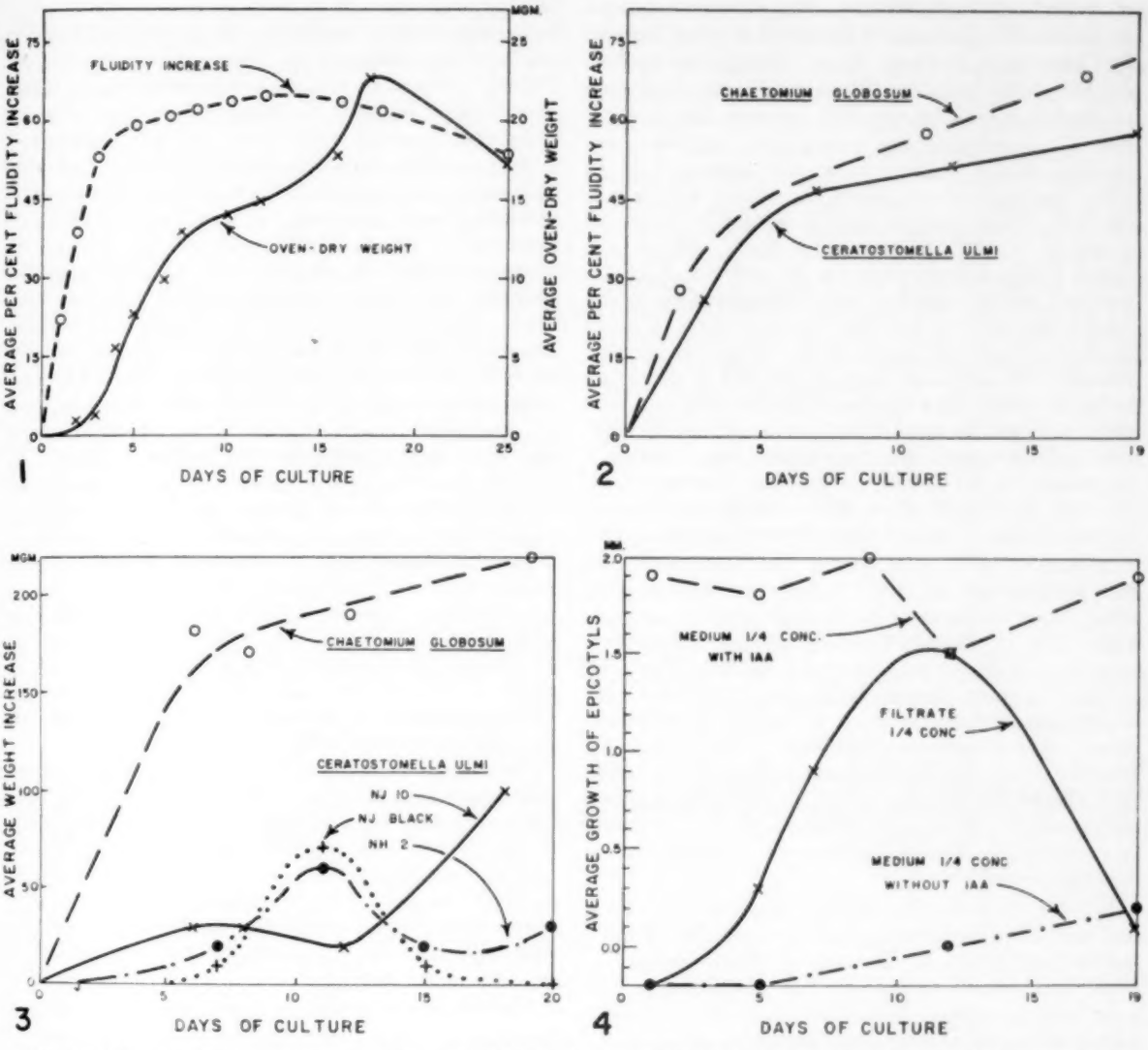

FIG. 1-4. Fig. 1. Average percentage increase in fluidity of citrus pectin induced by culture filtrates and average oven-dry weight yield of mycelium in relation to culture period.—Fig. 2. Average percentage increase in fluidity of methyl cellulose induced by culture liquids of *Certostomella ulmi* and of *Chaetomium globosum* in relation to culture period.—Fig. 3. Average weight increase of alkali-treated cotton fiber following exposure to culture fluids of *Ceratostomella ulmi* and of *Chaetomium globosum* in relation to culture period.—Fig. 4. Average growth of etiolated pea epicotyls induced by culture fluids of *Ceratostomella ulmi* in relation to culture period.

tions. Pectic acid was used to provide a demethylated substrate for any polygalacturonase present. A 1 per cent solution of "Pectinol 100D"[7] was substituted for the filtrate in procedural checks. These solutions were tested for the presence of monogalacturonic acid after 3, 5, and 7 days of incubation at 23 ± 1° C. Chromatograms were allowed to develop for 24 hours at room temperature. In all cases, spot tests for monogalacturonic acid were positive with "Pectinol" (11 trials) but negative with filtrates of *C. ulmi* (22 trials). It was concluded, therefore, that deploymerase (DP), but not polygalacturonase (PG), was the pectolytic enzyme produced.

The effect of substrate on enzyme production was determined by supplying glucose and pectin as sole carbohydrate sources in the culture medium. Six-day-old cultures of 3 isolates were tested viscometrically. Average fluidity increases were 70 and 60 per cent for media that contained pectin and glucose, respectively. These results indicate that the presence or absence of pectin as a substrate has little effect upon production of this enzyme.

Cellulase production.—Cellulolytic activity of seeded culture fluids was measured by decrease in viscosity

[7] Rohm and Haas Company.

of methyl cellulose solutions. The decreases induced by isolates of *C. ulmi* were compared to those induced by *Chaetomium globosum* Kunze. The culture medium for both fungi contained 0.5 per cent methyl cellulose as the sole carbon source. Two per cent methyl cellulose (Dow) solution was prepared by dispersing the granules in hot distilled water and chilling to 5° C. After the solution was mixed thoroughly with a stirring rod, 10-ml portions were dispensed by means of a syringe into 125-ml Erlenmeyer flasks. These were cotton plugged, autoclaved for 20 minutes at 15 lb. pressure, cooled rapidly, and replugged with sterile rubber stoppers to minimize moisture loss. Five-ml portions of seeded media were added aseptically. Fluidity was measured immediately and at 4 and 24 hours thereafter. The apparatus was cleaned with cold water followed by alcohol and acetone rinses to minimize contamination. This experiment was repeated 3 times with the NJ "Black" isolate and once each with the NH2, NJ6, NJ10, RI-A, RI-B, and RI-C isolates of *Ceratostomella ulmi* and with *Chaetomium globosum*.

A marked increase in fluidity was found with culture solutions for all of the isolates of *Ceratostomella ulmi*. Since results were comparable, average data are given (Fig. 2). Fluidity increases indicate that enzyme titre increased most rapidly during the first 7 days of culture; however, growth during this period was generally sparse. Data were on the order of those obtained with *Chaetomium globosum*. Sterilization by heat destroyed activity whereas sterilization by filtration (Berkfeld) had no effect. These results suggest that a cellulolytic enzyme of the Cx type is produced in considerable amounts early in the growth of *Ceratostomella ulmi*.

In order to determine whether or not the substrate had any effect on enzyme production, the NJ "Black" isolate was grown for 18 days on basal medium in which dextrose or methyl cellulose was added as the only carbon source. Fluids of these cultures were added to methyl cellulose test solutions. Fluidity increases of 43 and 40 per cent were obtained after 4 hours' incubation with dextrose and methyl cellulose treatments, respectively. Therefore, substrate does not appear to have any effect on cellulolytic enzyme production.

Production of the cellulase complex was further studied by measuring the weight increase of alkali-treated cotton fiber following exposure to culture filtrates of 3 isolates of *C. ulmi* (NJ10, NJ "Black," and NH2) and *Chaetomium globosum*. Distinct increases in alkali centrifuge value were obtained with all isolates of *Ceratostomella ulmi* tested (Fig. 3). These were relatively small when compared to those obtained with *Chaetomium globosum*, however. Although no measurements were made, mycelial growth of *Ceratostomella ulmi* on cotton was sparse, whereas that of *Chaetomium globosum* was profuse. These data indicate that swelling of the cotton fiber had occurred and that a cellulase (probably of the C1 type) was produced to some degree by isolates of *Ceratostomella ulmi*.

Growth-regulator activity.—Etiolated pea epicotyl sections were exposed to culture fluids of the NJ "Black" isolate to detect growth-promoting activity. Results were variable; however, in 3 of 5 experiments conducted, a strong growth response was observed.

In 1 experiment, positive curvatures were obtained with split pea-sections. In 2 subsequent experiments, straight-growth data were obtained. In the first of these, 8-day cultured fluid produced an average increase in growth of 1.1 mm above that of nonseeded medium. Eleven-day cultures induced no growth or slight inhibition. Similarly, no growth occurred in 14-day cultured fluid, but an increase in growth of 1.5 mm occurred in a 1:3 dilution of this fluid.

In the final experiment, 1:3 dilutions of seeded and of nonseeded medium were used. Elongation of epicotyl sections increased with age of cultures. In 12-day-old cultures, growth exceeded that in checks by 1.5 mm (Fig. 4) as in the previous experiment. Growth approached that obtained by the addition of 5 ppm indoleacetic acid to nonseeded medium. These results indicate that a growth-promoting substance is produced by *C. ulmi* in culture. The fact that dilution of the culture fluids was necessary to obtain optimum growth response indicates that this stimulator is produced in considerable amounts. Additional work is needed to determine the nature of this substance and the conditions under which it may be produced.

Discussion.—Considerable evidence that fungal pectic enzymes cause vascular plugging and discoloration, especially in Fusarium and Verticillium wilts of tomato, has been reported (8, 13, 14, 19). Occlusion apparently results from the hydrolysis of pectic materials in the middle lamellae and primary cell walls. The resulting molecular fragments aggregate to form vascular blocks that reduce the water-carrying capacity of the vessels.

The abundant production of pectin depolymerase found in this study indicates that a comparable situation may obtain with gummosis in elms wilting from infection by *C. ulmi*.

Many wilting plants, including elm and oak, exhibit tylosis as well as gummosis in the vascular system (2, 20). A study by Chattaway (4) on gummosis and tylosis in heartwood formation may be pertinent. The occurrence of tylosis was associated with large pit size and gummosis with small pit size, whereas both gums and tyloses were found in plants having pits of intermediate size. She concluded, therefore, that both of these phenomena have a common basis of origin and that pit size determines the particular manifestation.

That pathological tylosis is not far removed from the normal physiological change in heartwood formation is indicated by the fact that both occur in ray parenchyma cells that lie adjacent to vessels (4, 15). Also, a modification of the normal metabolism of these cells, rather than a more drastic action, is indicated by the development of secondary thickening and even

of pits in tylosis of pathologically wilting oaks (15). This observation must indicate that the host cells were able to maintain a metabolism not far from normal.

A comparable situation may then obtain in both pathological and heartwood tylosis. Such an assumption would indicate a shift in metabolism such as an increase in carbohydrases that affect the primary cell wall. The production of 2 cellulases (of the C1 and Cx types) and of pectin deploymerase by *C. ulmi* may therefore be of some significance. The growth of *Endoconidiophora fagacearum* Bretz on pectin, xylan, and dextrin as sole carbon sources (1) indicates that this organism, too, is a strong producer of carbohydrases. Such enzymes could conceivably alter the metabolic balance of cells to set the stage for tylose formation.

To investigate this possibility, filtrates of *C. ulmi* were tested for growth effects on etiolated pea epicotyls. It was apparent that a substance comparable to indoleacetic acid in gross effect was produced in culture. The magnitude of response indicates that a growth substance may be involved also.

The presence of such a fungal growth regulator could have considerable importance in the disease mechanism. It is obvious that such a metabolite could play a part in tylosis. Furthermore, Thimann (17) states that "each auxin molecule causes the deposition of some 3×10^5 hexose residues as cellulose, as well as the pectin, hemicellulose, and protein, which also are laid down." The marshalling of the host cell protoplast to such a metabolism together with an equilibrium favoring hydrolysis of these materials, as presented by fungal enzymes, might provide a strong shunt metabolism. Such a shunt could greatly increase the occurrence of gummosis.

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ANGULAR LEAF SPOT OF BEAN[1]

C. Cardona-Alvarez[2] and J. C. Walker

SUMMARY

Of 18 species of legumes exposed to conditions favorable for infection by *Isariopsis griseola* Sacc., the incitant of angular leaf spot, only kidney bean and lima bean were affected. The fungus grew very slowly on a large number of culture media tested. Growth on agar occurred at 8°–28°C but not at 32°. Spore germination occurred from 8° to 32° but not at 36°. The optimum temperature for growth was 24°, and for germination the optimum range was 20° to 28°. Best growth on honey-peptone agar was at pH 5 and on potato-dextrose agar containing bean-leaf extract at pH 5 and 6. The pathogen invades through stomata of the leaf and progresses intercellularly through the mesophyll cells and the palisade layer. As necrosis of affected cells sets in, the fungus becomes intracellular and extends rapidly until it becomes restricted by vascular bundles. The fungus overwintered through 2 successive Wisconsin winters on infested plant debris. Infection occurred from 16° to 28° but not at 32°. Disease development was most rapid at 24°. Exposure of inoculated plants in a moist chamber for 3 hours was sufficient for infection, but the severity of the disease increased when the moist period was prolonged up to 24 hours. Coremial formation occurred only in humid atmosphere. The formation of the fruiting structure was completed in about 24 hours at 24° but heavy sporulation did not occur until 72 hours or longer. The spores were released in dry atmosphere and were disseminated readily by water- or wind-borne soil particles and by dry air currents and less readily by air-borne water. The most important climatic factors in production of epidemics are considered to be moderate temperature and rainy or highly humid periods sufficiently long to induce coremial formation and infection, alternating with dry atmosphere and wind action.

Angular leaf spot of bean (*Phaseolus vulgaris* L.), incited by *Isariopsis griseola* Sacc., is a common disease in tropical and subtropical regions. Although it has been regarded as a minor disease in the northern half of the United States, it sometimes occurs spasmodically in epidemic proportions. In central Wisconsin in 1954 it caused losses of 50 per cent or more in some fields. The disease affects stem, leaf, and pod. Grayish leaf lesions, which become light brown with age, assume final angular shape because of limitation by veins. Premature defoliation is common. Pod lesions are roughly circular and reddish brown with dark brown borders. Stem lesions are dark brown and elongate. On all lesions, dark stromata appear in considerable numbers; in moist weather macroscopically visible coremia are formed on the stromata. Miles[3] has described the morphology of the coremia and of the conidia. Symptoms of the disease are illustrated in Figure 1.

The present study is concerned with the host range and physiology of the pathogen, its relation to the host tissue, and various aspects of the disease cycle.

Host range.—During the growing seasons of 1951 to 1954, inclusive, at Medellin, Colombia, single rows of susceptible beans were planted alternately with double rows of the following species of legumes: *Phaseolus lunatus* L., *Dolichos lablab* L., *Dolichos* sp., *Indigofera sumatrana* Gaertn., *Cajanus* sp., *Canavalia gladiata* DC., *Canavalia ensiformis* DC., *Mucuna* (*Stizolobium*) sp., *Crotolaria* spp., *Pueraria javanica* Benth., *Pueraria* sp., *Clitoria ternatea* L., *Medicago sativa* L., *Soja max* (L.) Piper, *Vigna sinensis* (L.) Endl., and *Vigna* sp., *Melilotus alba* Desr. Although severe natural infection occurred in each season on bean (*Phaseolus vulgaris*), the only other species that showed symptoms of the disease was lima bean (*P. lunatus*). Although several varieties of soybean (*Soja max*) were included, no evidence of disease was noted, which is contrary to the report of the disease on this host in Siberia[4].

Isolation and pathogenicity of the fungus.—The fungus is one that is not readily isolated from host lesions by the usual tissue plating technique. A modification of the method developed by Brock[5] was used. A suspension of spores from naturally infected plants was sprayed upon the foliage and stems of young bean plants, which were placed in a moist chamber at 24°C for 5 days and then moved to a greenhouse bench at the same temperature. When typical lesions had formed, sections of diseased stems about 1 cm long were placed in 1:1000 mercuric chloride for 3–4 minutes, washed in sterile distilled water 6–8 times, and transferred to sterile Petri plates containing moistened filter paper. After 5–8 days at 24°, spores were transferred from the coremial tips to potato-dextrose agar slants. When colonies formed, a spore suspension was made and spread over the

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[3] Miles, L. E. 1917. Some diseases of economic plants in Porto Rico. Phytopathology 7: 345-351.

[4] Abramoff, I. N. 1931. (Title in Russian). Diseases and pests of soybeans in the far East. Vladivostock Far-Eastern Plant Protect. Sta. 120p. (Abs. in Rev. Appl. Myc. 11: 87).

[5] Brock, R. D. 1951. Resistance to angular leaf spot among varieties of beans. Jour. Austral. Inst. Agr. Sci. 17: 25-30.

surface of a Petri dish containing 2 per cent water agar. Individual germinated spores were transferred to slants of potato-dextrose agar containing 1 per cent bean leaf extract (PDAB).

Inoculum was prepared from 10- to 20-day cultures on PDAB agar in Petri dishes. The fungus mycelium and spores from several plates were removed and homogenized in a Waring Blendor in 200 ml of distilled water. After the coarser material had settled, the supernatant liquid was diluted and sprayed on plants with an atomizer. Inoculated plants were kept in a moist chamber at 24°C for 48 hours then removed to a greenhouse bench at the same temperature. Typical symptoms appeared in about 10 days. In tests with plants ranging in age from 10 to 60 days after planting, all plants passed through the usual sequence of spotting, necrosis, chlorosis, and defoliation, regardless of age of the plants at the time of inoculation.

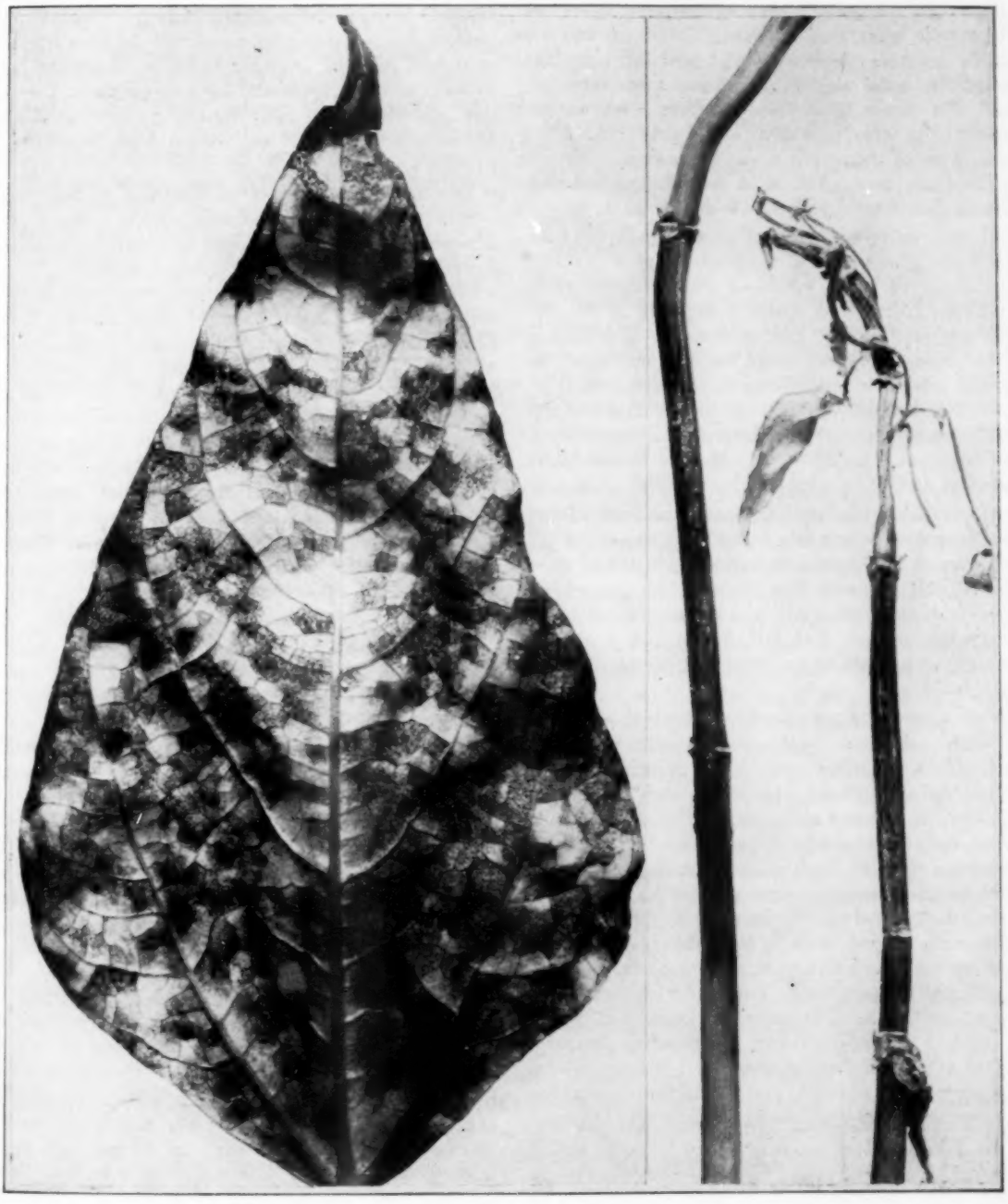

FIG. 1.—Lesions of angular leaf spot on leaf and stem of bean.

PHYSIOLOGY OF THE FUNGUS.—The fungus was grown on yeast-extract agar with and without supplements of mineral salts and sucrose; on coconut-milk agar with and without supplements of glycine, thiamine, mineral salts, and sucrose; on Nutramigen[6] agar; on casein-hydrolysate agar supplemented with a mixture of thiamine, riboflavin, pyridoxin, pantothenic acid, *p*-amino benzoic acid, nicotinic acid, choline, inositol, biotin, folic acid, nucleic acid, and sucrose; on potato-dextrose agar with and without extract of barley leaf and bean leaf; on barley-leaf agar; on oat-leaf agar; on cornmeal agar; on oatmeal agar; on honey-peptone agar; on Richard's solution; and on Richard's solution agar with and without bean-leaf extract. The solid media in Petri dishes were implanted with 5-mm discs removed from a culture on potato-dextrose agar plus bean-leaf extract (PDAB). Growth at room temperature was exceedingly slow on every medium used. On solid media, the colonies reached a diameter of only 15–20 mm in 25 days.

Petri dish cultures on honey-peptone agar and on PDAB were grown at 4°C intervals from 8° to 36°. Growth occurred at 8°, increased with temperature to an optimum at 24°, and declined abruptly at 28°. No growth occurred at 32° and 36°. Spore germination tests on deep-well slides were carried out over the same temperature range. Germination occurred at 8° and was accelerated (percentage of germination and growth of germ tubes) with increase in temperature to an optimum at 20°–28°. Meager germination occurred at 32°.

Petri dishes of honey-peptone agar and of PDAB, both adjusted with suitable buffer to a range of pH values from 3 to 9, were implanted with 6-mm discs from a PDAB culture. The optimum for growth on honey-peptone agar was pH 5 and with PDAB maximum growth was at both pH 5 and pH 6. Slight growth on each medium occurred at both extremes of the pH range.

HOST-PARASITE RELATIONS.—Leaves of Idaho Refugee bean were inoculated by depositing, with a Pasteur pipette, drops of spore suspension in reinforcement rings located on the lower side of the leaf. The plants were placed in a moist chamber at 20°C for 48 hours and then removed to the greenhouse bench at the same temperature. For the study of penetration, leaves were cleared in a saturated aqueous solution of chloral hydrate, dehydrated in 3 changes of 100 per cent ethanol, and stained with 1 per cent fast green in 95 per cent ethanol, the excess stain being removed with 100 per cent ethanol. For study of the fungus following penetration, sections of inoculated leaves were fixed in a 5-5-90 mixture of formalin, propionic acid and ethanol under vacuum for 1–3 hours, dehydrated in tertiary butanol, and embedded in paraffin. Ten-μ sections were fixed to slides with Haupt's adhesive and stained with Conant's quadruple stain.

Penetration occurred through stomata. As the fungus developed in the substomatal cavity, chloroplasts of cells adjacent to the cavity stained red in contrast to the green coloration of normal chloroplasts. At 3 days, necrosis of the guard cells and adjoining mesophyll cells occurred, and chloroplasts showed signs of disintegration. As the fungus progressed intercellularly, disintegration extended to the spongy parenchyma, to the palisade cells, and finally to the upper epidermal cells. At about the ninth day, the fungus became intracellular in the necrotic and immediately surrounding tissue, its advance becoming restricted only by vascular bundles. Stromata began to form in the substomatal cavities on about the tenth day, and they were fully formed by the twelfth day. Coremia were formed only when high humidity was provided.

THE DISEASE CYCLE.—*Overwintering of the fungus.*—The means of overwintering of the fungus under Wisconsin conditions in seed, in debris of previously infected plants, and in soil were studied. To test survival on or in bean seeds, tests were made with the following lots of seed: 1) seed from healthy plants; 2) healthy seed sprayed with a spore suspension and allowed to dry; 3) seed harvested from a field of infected plants at Babcock, Wisconsin, in 1954; and 4) seed from diseased pods of plants inoculated in the greenhouse. The seeds were planted in sterilized soil in sterilized pans, covered with sterilized quartz sand, and watered daily. After the seeds had germinated, the pans were placed in a moist chamber at 24°C for 12 days and then removed to the greenhouse bench at the same temperature. As controls, seedlings were sprayed with a spore suspension and kept in environments similar to those used for the test plants.

After 42 days, no evidence of disease appeared on plants from any of the lots of seed although control plants were severely affected. In another trial, seeds from infected pods were allowed to germinate on filter paper in a sterile moist chamber; in another moist chamber, healthy seedlings were sprayed with a spore suspension. After 30 days, no evidence of disease was noted in the test seedlings, although the control seedlings were severely diseased.

Infected leaves, stems, and pods from artificially inoculated plants and from naturally infected plants collected at Babcock in September, 1954, were placed in cheesecloth bags, some of which were exposed to winter climate at Madison, Wisconsin, and others were kept at room temperature and humidity in the laboratory. Portions of each lot were pulverized and suspended in water and used as inoculum at monthly intervals beginning 5 months after collection. Additional debris from infected plants was collected in the field at Babcock in April, 1955, and tested also at monthly intervals. With each lot of material, inoculated plants became infected over a period of 19 months after the autumn collection of 1954. The amount of infection was always less on plants inocu-

[6] Nutramigen contains casein hydrolysate, maltose, dextrins, fat, minerals, and vitamins. It is manufactured by Mead Johnson and Co., Evansville, Indiana.

lated with material exposed out of doors than on those inoculated with material left in the laboratory.

Spores from coremia on the material used in the tests just described were tested periodically. The percentage of germination approached 100 per cent at the beginning of the tests. When the material was kept in the laboratory, germination declined steadily to about 10 per cent at 150 days, 2 per cent at 300 days, and none at 360 days. In material overwintered out of doors, the germination had dropped to about 1 per cent by April, 1955. When overwintered leaves were placed in moist chambers, coremia were produced up to 9 months after collection, but not after a longer period. When stems were kept in the laboratory, coremia formed after 12 months but not after 15 months.

Soil samples were collected in April, 1955, from a field where the bean plants were heavily infected the previous season. The soil was screened to remove plant debris, suspended in water, and used for the inoculation of plants. No disease developed. Another test was made by mixing infected leaves, stems, and pods with soil in the autumn of 1954 and exposing the mixture to winter weather at Madison until March, 1955, when the debris was removed and a soil suspension used as inoculum. No disease developed.

It is evident from these studies that the fungus may overwinter under Wisconsin conditions in infested plant debris. Although a few conidia may survive, it is not likely that they are important as primary inoculum in the spring. Since the fungus is dependent upon viable conidia for primary infection, the stromata are the most important overwintering phase. Under proper conditions, they produce new coremia and conidia that comprise the primary inoculum. Seed and soil were not shown to be vehicles of the overwintering fungus.

Temperature relations.—The effect of temperature on disease development was studied in a series of experiments in which inoculated plants were exposed to temperatures at 4°C intervals from 16° to 32°, inclusive. The inoculum was prepared by grinding in a Waring Blendor the mycelium and spores from a 10-day culture on PDAB. Twenty-day-old plants were inoculated on the first 2 trifoliate leaves after removal of the unifoliate leaves. The inoculated plants were distributed at random in 5 groups of 8 plants each. Each group was placed in moist chambers at 1 of the 5 temperatures indicated. After 48 hours, they were removed to greenhouse benches at the same temperatures. Disease severity was recorded as the portion of leaf area covered by lesions according to the scale of Peterson et al.[7] The disease developed most slowly at 16°; the first symptoms appeared at 12–15 days (Table 1). The incubation period was 10 days at 20° and 28°, and 9 days at 24°. No infection occurred at 32°. At each temperature where infection occurred, the disease appeared as small yellow spots that became necrotic and angular. At 16°, spots enlarged and coalesced with age, sometimes becoming zonate with a greenish tinge. Necrosis developed more rapidly at higher temperature than at lower ones. Defoliation began first and was completed most rapidly at 24°, whereas it appeared latest and was completed most tardily at 16°.

TABLE 1.—*Incubation period, disease severity, and rate of defoliation in bean plants inoculated with Isariopsis griseola and then held at different air temperatures*[a]

Temperature °C	Incubation period (days)	Degree of disease severity[b] after			Defoliation period (days after inoculation)		
		15 days	18 days	20 days	Began	Completed	Length
16	12-15	7.9	19.3	35.5	26	45	19
20	8-12	39.2	49.2	53.9	18	30	12
24	8-10	62.9	76.0	81.0	14	24	12
28	8-12	28.7	42.5	47.2	16	28	12
32	No infection	0	0	0			

[a] Average of 5 experiments; 8 plants each treatment; observations on 6 leaflets of each plant.

[b] The values are averages of ratings made according to scale of Peterson et al.[7] A value of 100 represents a condition in which approximately 37 per cent of the leaf area is occupied by lesions.

Moisture.—Plants were sprayed on the first trifoliate leaves with a spore suspension and placed in a moist chamber at 24°C for various intervals. When the plants were taken from the chamber, the moisture was removed immediately by placing them in front of an electric fan until dry. They were then returned to the greenhouse bench. In each experiment, 1 set of plants was dried immediately, without exposure in the moist chamber. In the first experiment, plants were removed at daily intervals for 13 days. In the second experiment, plants were removed at daily intervals for 6 days. In the third experiment, they were removed at 3-hour intervals for 24 hours. A few lesions developed on unexposed plants. In the first 2 experiments, all exposed plants became severely diseased. In the third experiment, the average numbers of lesions per plant with exposures of 0, 3, 6, 9, 12, 15, and 24 hours were 1.6, 6.4, 9.5, 17.8, 51.6, 65.5, and 130, respectively.

Plants were exposed to high moisture at 24°C for various periods previous to inoculation. They were removed from the moist chamber, sprayed immediately with inoculum, and returned to the chamber for 24 hours. They were then removed to the greenhouse bench at the same temperature. Preinoculation exposure periods were 24-hour intervals for 1 to 6 days, inclusive. Control plants without preinoculation moist treatment were included. No difference between plants without preinoculation treatment and those with various intervals of treatment was observed.

[7] Peterson, R. F., A. B. Campbell, and A. E. Hannah. 1948. A diagramatic scale for estimating rust intensity on leaves and stems of cereals. Canad. Jour. Res. Sect. C. 26: 496-500.

Throughout all the studies reported above, no coremial formation was observed if the inoculated plants were removed from the moist chamber before symptoms were visible. In order to determine the relation of moisture to coremial formation, diseased leaves and stems on which stromata were present were incubated at 24° in sterilized Petri dishes provided with moist filter paper. The material was examined at frequent intervals. At 3 hours, young coremia were raised and conical in shape as the stromatic mass pushed through the stoma. At 6 hours, a few hyphae had started to diverge at the tip of the coremium. At 8 hours, the coremia were nearly completely formed and more hyphae were divergent. At 24 hours, the coremium was composed of a bulbous stromatic base, a median constricted neck of brown septate hyphae that were densely packed, and a mass of divergent hyphal tips. At 48 hours, spores had begun to form as swellings at or just below the hyphal tip. Heavy sporulation did not occur until 72 or 96 hours and occasionally not until 144 or 168 hours. The response of the fungus in necrotic lesions on green leaves was similar to that in older chlorotic leaves. It was slightly slower on stem lesions. When infected host material was removed to a dry atmosphere, sporulation was checked completely.

Light intensity and duration.—Plants were inoculated and exposed to moist treatment for 48 hours, then groups were placed in a series of chambers adjusted to approximately 650, 430, 330, and 250 foot-candles for 16 hours each day during the incubation period. In another experiment, the photoperiods after the postinoculation treatment were set at 4, 8, 12, and 16 hours per day. There was little difference in incubation period and rate of defoliation in plants subjected to the different light-intensity treatments, although necrosis was most severe at the 2 intermediate light intensities. In the photoperiod treatments, the disease was most severe at the 12-hour light period and least severe at the 4- and 16-hour treatments.

Dissemination.—In 2 experiments, the effect of splashing of water from soil to foliage on dissemination of the pathogen was studied. Seeds were planted in sterilized soil in galvanized pans. When the seeds had germinated, a spore suspension was applied to the top layer of soil. Subsequent watering of the plants was varied so that splashing of water and soil particles on stems and lower leaves occurred in one lot and splashing was avoided entirely in the other lot. The pans were then placed in a moist chamber for 2 days at 24°, then removed to the greenhouse bench. A third control lot was sprayed with a spore suspension and exposed to the same moisture period. In the lots in which splashing of soil occurred, 100 per cent of the plants showed stem infection and 12 per cent of the leaves were infected in the first experiment; in the second experiment, 68 per cent of the stems and 56 per cent of the leaves became infected. When splashing was avoided, the plants remained symptomless in both experiments. In both cases the control plants were severely diseased.

The possibility of dissemination of the fungus by wind-blown soil particles was studied. Soil in which bean plants were growing in a pan 70 × 40 cm in surface area was infested by pouring a spore suspension over the surface of the soil. When the soil had dried on the surface, an air current from an atomizer was directed toward the soil so that soil particles were carried to healthy plants located at different distances. After 48-hour exposure, the plants were placed in a moist chamber for 48 hours and then removed to the greenhouse bench. Plants within a distance of 2 m became infected.

Several pots of infected plants on which the fungus was sporulating were arranged in a triangle, and a similar triangle of pots of healthy plants was placed so that the triangles faced each other at one of their respective acute angles. An air current was directed at the base of the infected group in the direction of the healthy group. Other pots of healthy plants were placed at random in the greenhouse up to a distance of 9 m. After 48 hours, the plants were placed in the moist chamber for 48 hours and then returned to the greenhouse bench. In each of 3 experiments, plants located up to a distance of 7 m from the source of inoculum became infected. When the experiments were repeated 3 times with atomized water instead of air alone, only those plants located up to 2 m from the source of inoculum became infected. When coremia became moistened, they bent over to the leaf surface to form a compact mass from which spores were not released readily. This condition undoubtedly accounted for the less effective dissemination of spores by atomized water.

On the basis of these experiments, it was concluded that wind-blown particles of recently infested soil, wind-blown spores, and water-borne spores are all effective agents of dissemination.

Discussion.—Since the epidemiology of angular leaf spot of bean is poorly understood, this study was directed toward securing more information concerning environmental and certain other factors in relation to the development of this disease. Although the world distribution of angular leaf spot has led to its being regarded generally as a tropical and subtropical disease, its spasmodic occurrence in temperate regions shows that it is not restricted to warm climates. No evidence was secured that the pathogen is carried in or on seed nor that it survives as a soil saprophyte. The fact that it survived in infested plant debris for 19 months including 2 successive Wisconsin winters indicated that once established it subsists in this manner. Other hosts are not regarded as important, since only bean and lima bean were found to be susceptible.

The fungus grew in culture from 8° to 28°C but not at 32°, and spores germinated from 8° to 32°. The optimum for both processes was about 24°. Only a short exposure (3 hours) to moist conditions was necessary for minimal infection, but the latter was enhanced with prolonged moisture up to 24 hours.

Once penetration had occurred, disease development proceeded in relatively dry atmosphere and stromata formed abundantly in substomatal cavities; however, high humidity for 24 hours was required to complete coremial formation and a period of 48 hours or more was necessary for abundant sporulation. Infection occurred and disease developed rapidly from 16° to 28° with an optimum at 24°. No infection occurred at 32°. There is no evidence, therefore, that temperature is a limiting factor in temperate regions.

The most critical factor in production of epidemics in tropical and temperate zones appears to be moisture. Although rain, dew, or high humidity was essential for infection, relatively long moist periods were needed for sporulation. Once spores were formed, low humidity was favorable to spore release from coremia and to dissemination. The most favorable climatic complex for epidemic development of the disease, therefore, includes moderate temperature and high humidity (or a water film on infected foliage and stems) for 48 hours or more, alternating with periods of low humidity and wind action.

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PHYSIOLOGIC RACES OF THE BURROWING NEMATODE[1]

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SUMMARY

Field observations indicated the existence of 3 physiologic races of *Radopholus similis* (Cobb) Thorne. These races differ in ability to parasitize roots of citrus and banana. Laboratory evidence confirmed the existence of 2 physiologic races; one race parasitizes banana roots and the other parasitizes the roots of both citrus and banana. Adult female nematodes from both physiologic races appeared not to differ in gross morphologic dimensions and proportions.

The existence of physiologic races within species of plant parasitic nematodes has been recognized for some time. Discussions of this topic have been given by Goodey[2] and by Filipjev and Shuurmans-Stekhoven.[3] The burrowing nematode, *Radopholus similis* (Cobb) Thorne, which causes the spreading decline disease of citrus,[4] parasitizes many sub-tropical fruit trees and ornamental plants, including banana.[5] In Florida, clumps of banana (*Musa paradisiaca* var. *sapientum* Kuntze and *M. nana* Lour.) are often planted next to citrus groves bordering lakes, marshes, irrigation ponds, or drainage ditches. These banana plants, if parasitized, constitute potential reservoirs of infection for citrus. Therefore, it becomes important to know if physiologic races of burrowing nematodes occur in nature and whether or not the burrowing nematodes from banana will parasitize citrus. The present paper deals with this problem.

METHODS AND RESULTS.—The first evidence indicating the existence of physiologic races of *Radopholus similis* came from the observation that roots of banana (*Musa nana*) adjacent to a citrus grove were heavily parasitized whereas the citrus roots were not. This location was sampled 4 times during the following year, and the citrus roots always were found free of burrowing nematodes, although the citrus roots were intermingled with the infected banana roots.

Burrowing nematodes from this collection appeared to be identical morphologically with those causing spreading decline of citrus. In the following discussion, burrowing nematodes that parasitized banana roots only will be referred to as the "banana race."

Clumps of banana in or near 39 citrus groves, where banana plants were close enough to citrus for root contact, were sampled to find additional colonies of the banana race of burrowing nematodes. The burrowing nematode had parasitized banana but not citrus roots in 9 of these locations, both banana and citrus in 4, only the citrus roots in 3, and neither banana nor citrus in 23 locations. These data collected in the field indicated the existence of 3 physiologic races of burrowing nematodes separable by their ability to parasitize either banana or citrus, or both. Parasitized banana plants appeared not to be injured, whereas all infected citrus had symptoms of spreading decline.

Following the discovery that some collections of *R. similis* in nature parasitized banana roots but not

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[2] Goodey, T. 1933. Plant parasitic nematodes. Methuen, London. 306 p.

[3] Filipjev, I. N., and J. H. Schuurmans-Stekhoven, Jr. 1941. A manual of agricultural helminthology. E. J. Brill, Leiden. 878 p.

[4] Suit, R. F., and E. P. DuCharme. 1953. The burrowing nematode and other parasitic nematodes in relation to spreading decline of citrus. U. S. Dept. Agr. Pl. Dis. Rptr. 38: 379-383.

[5] Brooks, T. L. 1954. Host range of the burrowing nematode internationally and in Florida. Fla. State Hort. Soc. Proc. 67: 81-83.

citrus roots, attempts were made to reproduce this phenomenon under controlled conditions. In a preliminary test, sour orange (*Citrus aurantium* L.) seedlings were inoculated with the banana race. These nematodes did not parasitize the sour orange seedlings. An experiment was then made in which banana (*M. paradisiaca* var. *sapientum*) and rough lemon (*C. limon*) were cross inoculated with 2 collections of burrowing nematodes, one was the banana race and the other was from citrus.

Each of 6 containers was filled with 5 quarts of soil and roots from a burrowing-nematode-infected clump of banana; 6 similar containers were filled with soil and roots from infected citrus. Nematode-free rough lemon seedlings were planted in all containers, and nematode-free banana sprouts were planted next to the rough lemon seedlings in 3 containers of each series. These plants were grown at a constant soil temperature of 75°–78° F. After 6 months, all plants were examined for evidence of infection. The burrowing nematodes from banana infected the banana plants but not the rough lemon seedlings. In contrast, the burrowing nematodes from diseased citrus trees readily infected both citrus and banana roots.

In a second cross-inoculation test, nematode-free sour orange seedlings and banana plants (*M. paradisiaca* var. *sapientum*) growing in steam-sterilized soil were divided into 3 series of 6 sour orange and 6 banana plants each. Each plant in one series was inoculated with 200 burrowing nematodes collected from the same clumps of bananas as in the preceding experiment, each plant in the second series was inoculated with 200 burrowing nematodes collected from a spreading-decline-affected citrus grove, and plants in the third series were left noninoculated. The test plants were grown in soil maintained at 75°–78° F and examined for burrowing nematode infection after 3 and 6 months. The burrowing nematodes from the bananas infected the banana plants but not the sour orange seedlings; the citrus race of burrowing nematodes from a spreading-decline-affected citrus grove infected both the sour orange and the banana test plants. These experiments confirmed the existence of 2 of the 3 physiologic races found in the field.

Once it became evident that physiologic races of burrowing nematodes existed, adult females from the same sources used in the cross-inoculation experiments were compared for gross morphological differences. One hundred female burrowing nematodes of the banana race and 100 of the race attacking both banana and citrus were measured with an ocular micrometer. No differences were detected (Table 1), an indication that the adult females of the 2 races are morphologically similar if not identical.

TABLE 1.—*Morphologic dimensions and proportions for 2 "physiologic races" of female burrowing nematodes*

Dimension or proportion	Citrus race		Banana race	
	Average	Range	Average	Range
Body length in μ	665	552-800	674	536-848
Body width in μ	25.8	19.9-33.2	25.5	18.2-33.2
a[a]	25.8	20.4-33.3	26.5	21.5-34.9
b[b]	4.5	3.7- 6.1	4.7	3.5- 6.1
c[c]	9.3	6.2-12	9.4	6.8-13.8
Vulva[d]	57.6	49.0-65.5	56.0	48-65
Stylet length in μ	18.1	16.6-21.5	17.4	14.9-19.9

[a] Length of body divided by greatest body width.
[b] Body length divided by oesphagus length.
[c] Body length divided by tail length.
[d] Location of vulva from anterior end expressed as percentage of total body length.

DISCUSSION.—A physiologic race generally is understood to be morphologically identical with the species but to differ from it in some aspect of its physiology, such as parasitism. In this sense, 2 physiological races of the burrowing nematode (*Radopholus similis*) have been separated by differences in parasitic activity for citrus and banana. The existence of a possible third race, found in the field on citrus only, was not studied in the laboratory experiments undertaken. It is likely that other physiological races of this nematode could be detected by using additional species of host plants. At present, it is not known whether 1 or more physiological races of the burrowing nematode are involved in the spreading decline disease of citrus, but the existence of such races could readily explain some of the variation that occurs in the severity of disease in different groves. In the search for resistant citrus rootstocks, it will be necessary to test prospective plants against different populations of burrowing nematodes from numerous locations. Since one of the physiological races parasitized both citrus and banana, it becomes necessary to determine which race is present before deciding whether or not infected banana or other plants should be considered potential reservoirs of inoculum for citrus.

In Florida, quarantines have been placed against ornamental nurseries where plants were found to be infected with *R. similis* on the assumption that such plants could carry inoculum to citrus. Burrowing nematodes collected from several kinds of ornamental plants failed to parasitize citrus in exploratory tests. It is possible that the burrowing nematodes infecting some of these plants may be similar to the banana race. We know from other tests that the physiologic race causing spreading decline of citrus in Florida will infect *Citrus aurantium* L., sour orange; *C. sinensis* (L.) Osbeck, sweet orange (var. Pineapple); *C. limon* (L.) Burm. var. rough lemon; *C. paradisi* MacFad., grapefruit (var. Duncan); *C. reticulata* Blanco, tangerine (var. Dancy); *Persea americana* Mill., avocado; *Malpighia glabra* L., Barbados cherry; *Hedychium coronarium* Koenig, ginger lily; and *Musa paradisiaca* var. *sapientum* Kuntze, common banana.

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OAK STEM CANKERS INITIATED IN THE DROUGHT YEAR 1953[1]

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SUMMARY

Single or multiple canker-like lesions in the 1953 annual ring on the boles of young red oaks (*Quercus rubra* L.) and a few other forest-tree species seem to be associated with the severe drought of that year. Weather data and soil moisture measurements made with Bouyoucos blocks have established the severity of the 1953 drought in the area affected. No fungus recognized as causing cankers was isolated from the lesions, 59 per cent of which yielded no organism whatsoever. The oak species affected have previously been reported susceptible to drought injury. Ridge tops had a greater percentage of red oaks cankered than was the case on lower slopes. None of the red oaks found on alluvial bottomland were cankered. Suppressed trees were slightly but not significantly more cankered than dominant or codominant trees. Cankers were common on all sides of affected stems though significantly more were found on the south than on the east quadrant. Cankers were more frequently present and more abundant on twin trees than on single-stemmed trees. The condition was especially severe on the quadrants of twin stems adjacent to each other, where competition for a limited water supply would be the greatest.

The drought of 1953 caused severe and extensive injury to oaks in West Virginia. Many scarlet oaks (*Quercus coccinea* Muenchh) died singly and in groups, and smaller numbers of other oak species were killed. The greatest damage was done in the eastern counties where shallow soils are prevalent, but scattered trees died on ridge tops elsewhere. Since then, individual trees have continued to die or to die back from the tops, showing leaf discoloration patterns suggestive of water shortage, although 1954 and 1955 have not been particularly dry years. This persistent dying is not due to oak wilt, although a few such trees have become infected.[3] Some trees dying back from the top have exhibited bleeding cankers on the lower living portion of their boles.

In the fall of 1955, single and multiple cankers, constituting open and partially healed wounds, were noted chiefly on the boles of red oaks (*Q. rubra* L.) 2–12 in. in diameter occupying the high flat-topped Chestnut Ridge area of Coopers Rock State Forest near Morgantown (Fig. 1, 2). A similar condition had been noted earlier in a ridge-top stand of scarlet oak in another part of the same forest. Some of the weaker trees in these stands had died, but no conspicuous mortality had resulted from the drought. Many red oaks with cankered stems were not affected by dieback in 1955 nor did they show leaf symptoms. Some of the affected areas on the trunks had earlier exuded some sap, but they had not exhibited the severe bleeding sometimes noted elsewhere.

Conditions described in this paper represent one facet of an extensive and complicated syndrome of injuries that seem to have resulted from the 1953 drought in West Virginia.

The purpose of this study was to determine whether the cankers were associated with drought and acute water shortage, as they appeared to be. Except for the attempted isolation of possible fungus pathogens, no attempt has been made to determine or to explain how this sort of localized killing was brought about.

THE NATURE OF THE INJURY.—The injuries that initiated the open or healing cankerlike wounds (Fig. 3, 4) had, in the Coopers Rock State Forest, resulted in the death of localized, often lenticular, areas of cambium. Death had occurred at or near the time when growth of the 1953 annual ring would normally have been completed. Callus folds initiated in 1954 by the living cambium at the edges of the dead tissues have since partially closed most of the wounds. The sapwood, and sometimes the heartwood as well, is darkened locally internal to the dead cambial surface of the 1953 annual ring. The swollen margins produced by the healing callus have given these localized wounds the appearance of cankers. Where cankers occurred singly they usually ranged from ½ in. to 1½ in. in width and were 3–5 times as long, vertically. Where several individual cankers formed close together, the tissues between them often died too, so that extensive killed areas were sometimes produced. By 1955, trunk suckers had grown out in the vicinity of some of the cankers.

Some trees exhibited small bumpy bark swellings (Fig. 5) rather than open or partially healed wounds. The killing in such cases had been confined to localized areas within the inner bark.

FACTORS RELATED TO CANKERING.—In order to determine if the single and multiple cankers of the tree boles were related to drought, studies were initiated to learn about 1) weather conditions and soil moisture for the general area, 2) species affected, 3) cankering in relation to topographic position, 4) cankering in relation to crown class, and 5) portions of trees most

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[2] Plant Pathologist, and Silviculturist, respectively, West Virginia Agricultural Experiment Station.

[3] Cultures made from such trees generally have failed to yield the oak wilt fungus. One tree with these symptoms, which was not cultured while alive, later produced the subcortical fungus mats typical of *Endoconidiophora fagacearum*, the cause of oak wilt.

FIG. 1-5. Fig. 1. Surface view of single lenticular canker showing crack in bark covering the dead area in the cambium. In the spring of recent years, such cracks have sometimes exuded moisture. This portion of the stem surface would be given a severity rating of 1.—Fig. 2. Multiple-cankered area. Sunken bark areas between individual canker cracks indicate areas of secondary killing. The portion of stem surface shown would receive a severity rating of 4.—Fig. 3. Sectional view of canker like that shown in Figure 1. The cambium appears to have died at or near the completion of the third annual ring (1953), and the next 2 annual rings of callus growth have almost completely closed the wound. The killed bark has been pushed out from the injured area by rolls of callus formed beneath.—Fig. 4. Section of large canker showing discoloration of sapwood below the canker and scattered vascular discoloration of the 1953 annual ring. The growth of the 1953 ring was complete or nearly so when the cambium died in the area of the canker. Note the dark dense "drought ring" that is shown above the canker as the outermost layer of the 1953 ring.—Fig. 5. Surface view of bumpy bark swellings not associated with cracks or dead cambium but rather with localized areas of bark necrosis.

seriously affected.

WEATHER CONDITIONS AND SOIL MOISTURE.—Precipitation and temperature records were obtained from the nearest weather station, Brandonville (6, 7), approximately 8 miles east of the study area. Although Brandonville is at a somewhat lower elevation, weather conditions are quite similar to those of Coopers Rock State Forest. As the cambium is believed to have died at or close to the time when the growth of the 1953 annual ring was completed, records for that growing season and for 1954 and 1955 are shown.

The summer precipitation during 1953 was considerably below normal, (Table 1) the summer being the driest since 1930. It was much drier than the summers of 1954 and 1955, during which no cankers were produced.

Maximum temperatures were high, 27 days of the 6-month period being at least 90°F. During 10 consecutive days of late August and early September, temperatures of 90°F or above were recorded. No precipitation occurred during this 10-day period or for the previous 17 days.

Soil-moisture values were obtained from Bouyoucos resistance blocks established in connection with a general study of soil moisture in the summer of 1953. These were placed in a forest-covered Dekalb soil, half way up the south slope where cankering was studied in relation to topographic position, and near the main study area, at depths of 2 in., 1 ft., and 2 ft. The blocks and soil were not calibrated, but a reading of 75,000 ohms is considered to represent the moisture content of the soil at which wilting occurs (wilting percentage) in accordance with the work of Bouyoucos (2).

TABLE 1.—*Normal monthly precipitation as compared with actual rainfall recorded in 1953, 1954, and 1955 for the months May through October, inclusive, at Brandonville, approximately 8 miles east of the study area*

	Precipitation in inches					
	May	June	July	August	September	October
Normal	4.46	4.86	5.14	4.79	3.79	4.06
1953	3.39	1.35	2.23	1.48	2.09	0.55
1954	2.93	4.16	2.73	12.23	2.30	7.78
1955	3.70	4.84	3.30	4.28	2.81	3.92

Figure 6 shows daily records of precipitation and of maximum temperatures from May 1 to October 31,

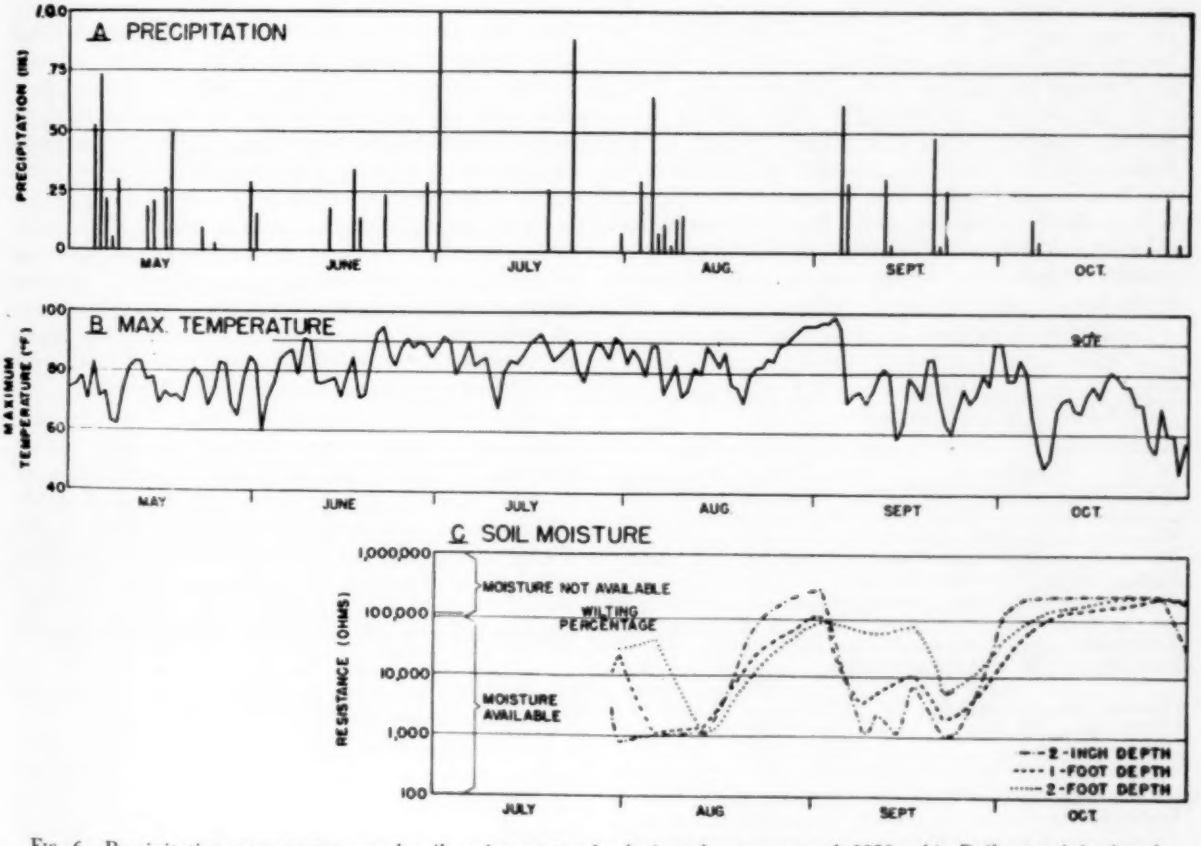

FIG. 6. Precipitation, temperature, and soil moisture trends during the summer of 1953. A) Daily precipitation in inches received at Brandonville, West Virginia. B) Maximum daily temperature in degrees Fahrenheit at Brandonville. C) Soil moisture trends at depths of 2 in., 1 ft., and 2 ft. in middle of slope in nearby Coopers Rock State Forest. Readings, in ohms, are from Bouyoucos resistance blocks.

1953, and periodic records of soil moisture from July 29 to October 31. One period of extreme soil dryness associated with extremely high air temperatures occurred in the latter part of August and early September. A second such period was recorded for most of the month of October.

Species affected.—This portion of the study was conducted on a 10-acre area where cankered trees were known to be abundant. This study area is a broad level ridge top having a dry rocky soil that is classified as Dekalb or Clymer, depending upon its depth. The site is subject to drought because of its location, and the abundance of large boulders and sandstones present throughout the soil body reduces the amount of pore space from which the well-stocked stand can obtain water. The stand is composed of mixed sprout hardwoods, 20–30 years of age, twins and multiple sprouts being common.

In a 6.7 per cent strip cruise on the 10-acre area, detailed data were taken on tree species, diameter and crown class, and the abundance of cankers by quadrants on single- and twin-stemmed trees. Cankers were found on 31.2 per cent of all trees (Table 2). It is evident that there is considerable variation in species susceptibility. Oaks were the most valuable species on the site. Red oak and black oak were the species most severely affected, with more than 50 per cent of the red oaks cankered. A higher percentage of the twin red oak stems were cankered than was the case for single-stemmed red and black oaks, the values being 60.2 and 52.2 per cent, respectively. This difference in degree of injury probably is related in part to the limited quantity of moisture available for 2 stems growing from a single root system or from competing root systems in the same soil area. It is of interest that chestnut oak and white oak showed very few and no cankers, respectively. Several species were unaffected by cankers on this site.

Cankering as related to topographic position.—A long, moderately steep slope, which had a southern exposure, and its adjacent alluvial bottom were selected for study to determine the relationship of topographic position to incidence of canker. Within 2 cruise strips, cankered and uncankered trees of the red oak group were tallied. The slope was divided equally into lower, middle, and upper portions for the purpose of analysis.

TABLE 2.—*Percentage of single and twin stems, by species, having 1 or more cankers*

Species		Total number	Percentage cankered
Common name	Scientific name		
Red oak	*Quercus rubra* L.	327	54.7
Black oak	*Q. velutina* Lam.	18	44.4
Black cherry	*Prunus serotina* Ehrh.	70	40.0
Cucumbertree	*Magnolia acuminata* L.	12	25.0
Red maple	*Acer rubrum* L.	28	14.3
Fire cherry	*Prunus pennsylvanica* L.f.	11	9.1
Chestnut oak	*Q. prinus* L.	106	1.9
White oak	*Q. alba* L.	56	0
Others[a]		93	0

[a] Big tooth aspen (*Populus grandidentata* Michx.), black birch (*Betula lenta* L.), black gum (*Nyssa sylvatica* Marsh.), black locust (*Robinia pseudoacacia* L.), dogwood (*Cornus florida* L.), hickory (*Carya* spp. Nutt.), sassafras (*Sassafras albidum* (Nutt.) Nees.), yellow-poplar (*Liriodendron tulipifera* L.).

TABLE 3.—*Percentage of cankered stems of the red oak group classified by topographic position*

Topographic position	Total number of stems	Percentage affected
Bottom (alluvial)	12	0
Lower ⅓	36	14
Middle ⅓	42	17
Upper ⅓	54	44

Cankering was most abundant on the upper slope and progressively less on those portions below; no cankers were found on the trees situated on the alluvial bottom (Table 3). The data clearly indicate that the canker was common on the dry (upper) portion of the slope, whereas few cankers were found on the more moist (lower) portions and none on the adjacent bottom. Observations over the general area confirm the association of cankers with dry sites. Since the data illustrated in Figure 6 were obtained from moisture blocks situated at a point midway between the lower ⅓ and the middle ⅓ of the cruised slope, the situation existing on the ridge top reflected in the degree of cankering shown in Table 3 is believed to have resulted from drought conditions even more severe than those recorded in Figure 6.

Cankering in relation to crown class.—The prevalence of cankers was studied by crown classes for stems of the red oak group on the broad ridge top. Perhaps the most outstanding fact is that a considerable amount of cankering occurred on stems of trees belonging to all crown classes and that cankering does not seem to be related to degree of dominance (Table 4).

Incidence and abundance of cankers on exposure quadrants of single stems.—Data were taken during the ridge-top cruise on the exposure distribution of cankers. Each single stem was considered to be divided into 4 quadrants whose centers were on the north, south, east, and west. Within each quadrant the abundance of cankers was measured in numerical severity ratings ranging from 0 to 4. The severely affected stems (rating 4) were so abundantly cankered

TABLE 4.—*Percentage of cankered stems of the red oak group classified according to crown class*

Crown class	Total number of stems	Percentage affected
Dominant	33	45.4
Codominant	152	55.9
Intermediate	64	43.7
Suppressed	100	61.0

that it was difficult to distinguish the separate cankers. Severity ratings 1 and 4 are illustrated in Figures 1 and 2, respectively. Although such a system is both arbitrary and subjective, the writers agreed closely on severity rating determinations in preliminary trials and all severity data were taken by them.

The degree of cankering incidence and severity was somewhat greater on the south sides of the stems than on any of the other 3 quadrants (Table 5). The difference between the mean severity rating for the south quadrant and that for the east quadrant was statistically significant at the 5 per cent level, but the difference between the figure for the south quadrant and that for the north or west was not, although significance was approached. Differences between figures obtained for canker incidence on different quadrants were not analyzed statistically.

The somewhat greater abundance of the cankers in the south quadrant may be related to greater water depletion within the stem on the south side. Trees growing in a well-stocked stand, having a uniform closed-crown canopy common to young even-aged stands in the region, should, on the average, have equal quantities of soil moisture available on all sides even in times of drought. If the south sides of the crowns tend to transpire more rapidly, the water depletion within the boles on the south quadrant may be greater than on the other sides of the stem.

Canker abundance on quadrants of twin trees.—The abundance of the canker injury on different sides of each twin stem was studied. The stem was considered to be divided into 4 quadrants, the centers of each quadrant being distinguished as (T) nearest its twin, (O) farthest from its twin, (R) right side, facing twin, and (L) left side, facing twin. The canker abundance was measured by numerical severity ratings in the same manner as for single stems.

TABLE 5.—*Incidence and abundance of cankers on north, south, east, and west quadrants of 174 cankered single stems (all species)*

Quadrant	No. with cankers	Percentage of quadrant cankered[a]	Severity rating[b] Sums	Mean[c]	Percentage[d]
South	145	83.3	245	1.41	27.7
North	130	74.7	217	1.25	24.6
West	130	74.7	211	1.21	23.9
East	120	69.0	201	1.16	22.8

[a] Percentage of quadrants cankered—100 times the number of times cankers were found on each quadrant divided by the total number of stems cankered.

[b] Severity rating—rated on a numerical scale of 0 to 4, with 0 indicating no cankers and 4 indicating that cankers were so numerous that separate cankers were difficult to distinguish.

[c] Mean severity rating—the sum of the severity ratings for a given quadrant divided by the total number of single stems cankered.

[d] Severity rating percentage—100 times the sum of the severity ratings for a given quadrant divided by the total of the sums of the severity ratings for all 4 quadrants (883).

The greatest abundance of cankers occurred on the quadrant nearest the other twin (Table 6). A statistical analysis showed canker abundance on this T quadrant to be significantly higher at the 5 per cent level than on the O, R, or L quadrants and nearly so at the 1 per cent level. Differences between the abundance of cankers found on quadrants O, R, and L were not significant. The significance of differences in canker incidence figures was not tested statistically.

It is probable that the portion of a twin stem nearest its twin suffers most from water deficiency in times of low available soil moisture and high transpiration. This is because the adjacent sides of twin stems often draw water from the vascular system of the same portions of the lower bole. In part, they take water from the same roots, and in part from roots occupying the same soil area as is occupied by roots of the other competing twin stem.

If this is so, the greater severity of the canker condition found in twin stems and its distribution with reference to the other twin emphasizes the basically physiological nature of the injury.

NO SPECIFIC FUNGI FOUND CONSTANTLY ASSOCIATED WITH CANKERS.—Attempts were made to isolate possible pathogenic fungi from the discolored sapwood behind the open cankers. Normal and pathological sapwood tissues were cultured from several cankered areas on each of 6 red oaks and from a dark vascular discoloration that extended as streaks in the sapwood above and below many of the large cankers (Fig. 4).

TABLE 6.—*Incidence and abundance of cankers on 50 cankered twin stems by quadrants*[a]

Quadrant	No. with cankers	Percentage of quadrant cankered[b]	Severity rating[c] Sums	Mean[d]	Percentage[e]
T	44	88	86	1.72	32.0
O	35	70	61	1.22	22.7
R	36	72	60	1.20	22.3
L	41	82	62	1.24	23.0

[a] The 50 stems were each individual stems of twin trees. Quadrants are designated as follows: T = quadrant nearest the other twin, O = quadrant farthest from the other twin, R = quadrant on the right as one faces the O quadrant, L = quadrant on the left as one faces the O quadrant.

[b] Percentage of quadrants cankered—100 times the number of times cankers were found on a given quadrant divided by the total number of twin stems (50) cankered on any quadrant.

[c] Severity rating—rated on a numerical scale of 0 to 4, with 0 indicating no cankers and 4 indicating that cankers were so numerous that separate cankers were difficult to distinguish.

[d] Mean severity rating—the sum of the severity ratings for a given quadrant divided by the total number of cankered twin stems.

[e] Severity rating percentage—100 times the sum of the severity ratings for a given quadrant divided by the total of the sums of the severity ratings for all quadrants (269).

About 300 chips were taken from normal and from pathological sapwood near the cankers. Five chips were cultured per petri dish of malt-extract agar medium.

Eighty-nine per cent of the chips taken from normal sapwood of cankered trees were sterile, 9 per cent yielded bacteria of various sorts, and miscellaneous fungi grew out from the remaining 2 per cent. Fifty-nine per cent of the chips taken from pathological sapwood yielded no microorganisms. Bacteria were cultured from 7 per cent. A *Fusarium* species grew out from 18 per cent and a pink *Cephalosporium* species from 5 per cent. The remaining 11 per cent of the chips from pathological sapwood yielded undetermined fungi and such common secondary organisms as *Trichoderma* and *Penicillium* species. No fungi recognized as wood-decay organisms were isolated.

Isolations made from the black vascular discoloration above or below the cankers usually yielded the same *Fusarium* species that was isolated from the discolored sapwood behind the canker.

Whereas it seems desirable to test the pathogenicity of the Fusarium and perhaps the Cephalosporium as well, there is no clear suggestion here that either organism is constantly associated with the cankered condition.

Possibly isolations made by other procedures or at other seasons may yield additional fungi. It is possible, too, that fungi might have acted under the extremely dry conditions but failed to survive within the wounds they helped to cause when normal weather conditions returned. If fungi did play some part in canker formation in the drought year of 1953, it would seem probable that their role was related chiefly to the localization of the injury.

Discussion.—The initiation of stem injuries resulting in single or multiple canker-like lesions on the boles of young red oaks and a few other forest tree species seems associated with drought, if not actually due to it. Extremely dry hot weather prevailed during the latter part of the 1953 growing season at or near the close of which the initial injuries are believed to have occurred. Drought occurred throughout the soil body twice during this period. Little or no water was available as determined from measurements made with Bouyoucos blocks.

Although fungus-caused canker diseases of trees have been reported to increase in incidence and severity as the result of dry weather (3), the failure to isolate any probable causal organism with regularity from the typical injuries suggests that the drought was more important in inducing the injuries described here than were the organisms isolated from these injuries. Perhaps causal organisms were fleetingly present but can no longer be isolated by the methods used, or perhaps the localized killing of the bark and cambium in time of drought may be explained on some purely physiological basis not yet understood.

The present paper calls attention to the existence of these cankers, to their initiation in the drought year of 1953, and to the critically low soil moisture resulting from inadequate rainfall associated with high temperatures that prevailed at that time. The oak species here affected have previously been reported sensitive to drought injury (1, 4). Trees of the red oak group suffered considerably, whereas white oak and chestnut oak were little affected. Ridge tops, generally acknowledged to be dry sites, had a greater percentage of trees cankered than did the lower slopes or alluvial bottoms. The fact that under the extremely dry soil conditions then prevailing the injury was not significantly more pronounced on suppressed trees, or on trees in the intermediate crown class, may be due to the protection from excessive transpiration afforded such trees by the crowns of those that overtopped them (5). The fact that cankers were common on all sides of the stems precludes the possibility that this injury is due to winter or to summer sunscald. Comparisons of canker incidence and severity on single-stemmed trees and twin trees indicate that cankers were most frequently found and most severe on twin trees. Here the competition of the twin stems for water, either within the vessels at the base of the shared bole, within the common root system, or within the soil in which the root systems of both compete for moisture would be expected to result in severe water shortage. On such twin stems, and especially on the stem quadrants of each adjacent to the other, cankering was significantly more frequent and more severe. The parts of the twin stems competing directly with each other for water suffered most from canker injury.

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YR CHARLESTON WAKEFIELD, A NEW YELLOWS-RESISTANT CABBAGE VARIETY[1]

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SUMMARY

YR Charleston Wakefield, a new variety of cabbage, conforms closely in season and horticultural characteristics to the variety Charleston Wakefield. It is homozygous for monogenic resistance to the yellows disease incited by *Fusarium oxysporum* f. *conglutinan* (Wr.) Synd. & Hans.

The pointed-head cabbage varieties used most widely in the United States are Early Jersey Wakefield and Charleston Wakefield, both of which were described by Boswell et al.[2] These varieties are used in areas where yellows (incited by *Fusarium oxysporum* f. *conglutinan* (Wr.) Snyd. & Hans.) occurs. A satisfactory resistant variety similar in type to Early Jersey Wakefield was introduced and described as Jersey Queen in 1932.[3] No resistant variety of the type of Charleston Wakefield was available until the development of YR Charleston Wakefield, which is described herein. This new variety was released to primary seed growers for increase in time for planting in 1954. Final release to the seed trade was made at the end of the seed crop season of 1955.

BREEDING PROCEDURE.—Charleston Wakefield differs chiefly from Early Jersey Wakefield and Jersey Queen in the following characteristics. The growing season is some 2 weeks longer. The foliage has slightly less bloom and is slightly lighter blue. The plants have greater height and spread of leaves, and produce larger heads. The tops of the heads are bluntly rather than sharply pointed, and the heads have a greater equatorial diameter in relation to the polar diameter than do those of the other 2 varieties.

The initial cross was made between yellows-resistant Jersey Queen and yellows-susceptible Charleston Wakefield. The F_1 plants were intermediate in type between the 2 parents. F_1 plants were selfed and F_2 progenies were grown on soil not infested with the yellows organism. Selection toward Charleston Wakefield type was made in the F_2 and F_3 selfed progenies. Seeds from the F_3 progenies were subjected to yellows-resistance tests.

Resistance to yellows in Jersey Queen is controlled by a single gene pair with resistance completely dominant over susceptibility.[3] This resistance is expressed in young seedlings and can be distinguished from susceptibility most readily by growing seedlings in soil infested with the yellows organism at a constant soil

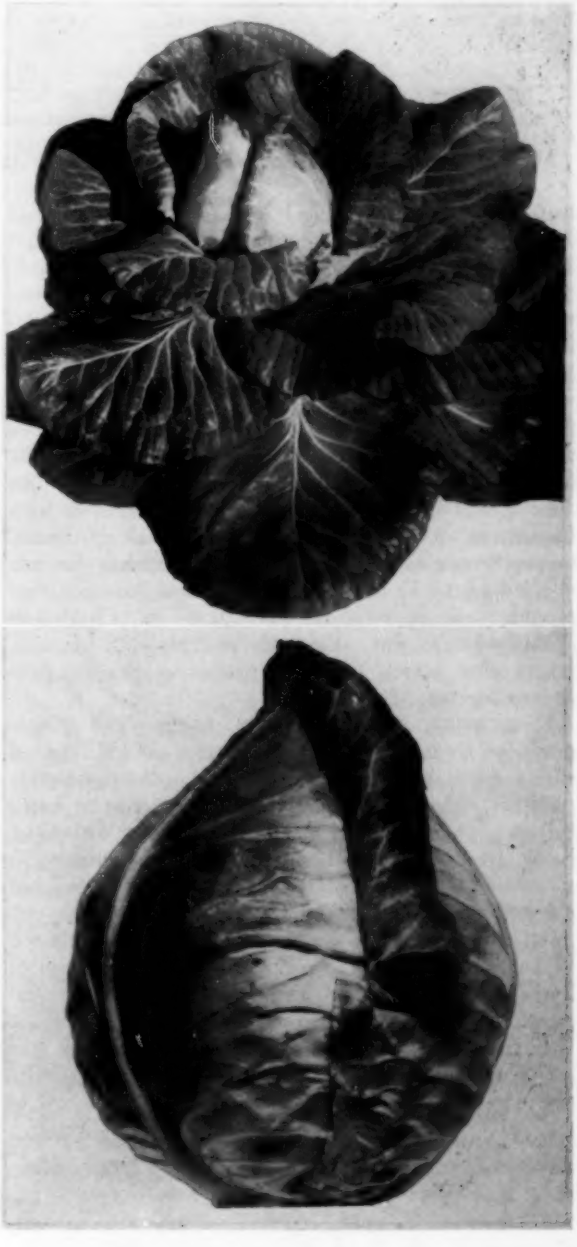

FIG. 1. Plant and head type of YR Charleston Wakefield.

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[2] Boswell, V. R., W. C. Edmundson, O. H. Pearson, J. E. Knott, C. E. Meyers, R. A. McGinty, W. H. Friend, H. H. Zimmerly, and J. C. Walker. 1934. Descriptions of types of principal American varieties of cabbage. U. S. Dept. of Agr. Misc. Pub. 169, 22 p.

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TABLE 1.—*Physical measurements of the cabbage varieties YR Charleston Wakefield, Charleston Wakefield, Early Jersey Wakefield, and Jersey Queen*[a]

Variety	Plant height	Plant width	Stem length from soil line	Weight of outer leaves	Weight of head	Ratio of head wt. to leaf wt.	Equatorial diameter of head	Polar diameter of head	Ratio of polar to equatorial diameter	Core length	Ratio of polar diameter to core length
	cm	cm	cm	g	g		cm	cm		cm	
Early Jersey Wakefield	28.2	59.9	7.4	677	1247	1.84	14.9	19.1	1.28	9.8	1.95
Jersey Queen	32.3	61.4	8.8	767	1368	1.78	15.6	19.9	1.28	10.6	1.88
Charleston Wakefield No. 1[b]	36.3	71.4	10.0	1184	2162	1.83	19.0	22.8	1.20	12.4	1.84
Charleston Wakefield No. 2[b]	35.8	70.2	10.2	1204	1955	1.62	18.1	20.1	1.11	10.5	1.91
Charleston Wakefield No. 3[b]	35.2	71.6	9.7	1219	2145	1.76	19.1	21.6	1.13	12.8	1.69
YR Charleston Wakefield	31.3	64.6	8.3	1035	2255	2.18	19.2	21.1	1.10	11.0	1.92
LSD 19:1	3.5	3.6	1.3	238	296		1.5	2.3		1.4	
99:1	5.0	5.1	1.9	338	421		2.1	NS		2.0	

[a] Values are averages from 10 plants taken at random from 25-plant plots arranged in 3 randomized replicates at Madison, Wisconsin.

[b] Lots 1, 2, and 3 of Charleston Wakefield are samples from 3 distinct commercial seed sources.

temperature of 24°C. Seed was sown in noninfested soil in the greenhouse, and seedlings were transplanted 3 weeks later to shallow metal pans containing soil heavily infested with the yellows organism. The pans were inserted in soil-temperature-control tanks maintained at 24°C. Readings were taken about 30 days later. As was to be expected, certain F_2 progenies were completely free from yellows, demonstrating that they were homozygous for resistance. These progenies were retained, and selection toward Charleston Wakefield was continued for 2 successive generations. The most promising individuals were then crossed with Charleston Wakefield, and selection was continued to the F_3 generation. The yellows-resistance test was applied to the F_3 progenies, and homozygous resistant progenies were retained. Selection for Charleston Wakefield type was continued for 2 successive generations after which the most promising progeny was chosen for mass increase.

EVALUATION AND RESISTANCE TESTS.—The plants produced by mass increase were tested on soil infested with the yellows organism and found to be completely resistant. Also, the plants were compared as to horticultural characteristics with Charleston Wakefield, Early Jersey Wakefield, and Jersey Queen during 3 successive seasons at Madison, Wisconsin, on soil not infested with the yellows organism. The plants matured at the same time as did those of Charleston Wakefield and about 2 weeks later than did those of Early Jersey Wakefield and Jersey Queen. Typical examples of plant and head type of YR Charleston Wakefield are shown in Figure 1. The foliage had less bloom and lighter color than the last 2 varieties and was indistinguishable in this respect from that of Charleston Wakefield. Certain physical measurements are given in Table 1. In some of these measurements, YR Charleston Wakefield resembled the resistant parent (Jersey Queen); in others, it resembled the susceptible parent (Charleston Wakefield). In plant height, plant width, and length of stem from the soil line to the base of the head, plants resembled Jersey Queen and Early Jersey Wakefield most closely. Weight of outer leaves and weight of head were not significantly different from those of Charleston Wakefield, and plants were distinct from all others in having a higher ratio of head weight to leaf weight. The head shape was characteristic of Charleston Wakefield. This was reflected in an equatorial diameter that was significantly greater than that of Jersey Queen or Early Jersey Wakefield and in a ratio of polar to equatorial diameter that was lower than that of either of the other 2 varieties. The ratios of polar diameter to core length of the 3 varieties did not differ consistently. In general, the deviations in type from Charleston Wakefield were minor and not disadvantageous. YR Charleston Wakefield should be a satisfactory replacement for Charleston Wakefield where yellows is a disease factor, and further tests may show that it should replace the latter variety generally.

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DEVELOPMENT OF THE ASCOGONIUM AND PERITHECIUM OF ENDOCONIDIOPHORA FAGACEARUM[1]

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SUMMARY

The development of the perithecium of *Endoconidiophora fagacearum* Bretz is considered to be initiated when a conidium, acting as a spermatium, unites with a trichogyne that extends to the surface from the base of the submerged ascogonium. The ascogonium originates from a lateral branch of the mycelium and at plasmogamy consists of 3 bulbous, greatly vacuolated cells and a septate branched trichogyne. Adjacent hyphae plus outgrowths from the ascogonium form a sheath around the ascogonial cells.

Spermatization is followed by a rapid multiplication of ascogenous cells that are cut off in chains from the 3 basal ascogonial cells. The ascogenous cells do not have definite cell walls. The basal cells of the ascogonium and the trichogyne were found to be multinucleate following spermatization.

About 20–30 hours after spermatization, sterile vacuolated cells develop among the ascogenous cells, and they are considered to exert an internal growth pressure and thereby to play a large part in the enlargement of the perithecium and in the elongation of the neck. The neck consists of an inner region of delicate, loosely associated cells and an outer region of closely associated hyphae that are continuous with the wall of the perithecium.

The ascogenous cells are unwalled throughout their existence but retain their form, probably by means of a plasma membrane. Two dikaryotic nuclei fuse to form a diploid nucleus characterized by a large nucleolus and nuclear vacuole. The nuclear membrane disappears and 3 divisions take place in the cytoplasm of the ascus, resulting in 8 ascospores that are elongate, hyaline, slightly curved, and uninucleate. The first ascospores were recognized 40–50 hours following spermatization, and they were formed first in the center of the perithecium.

Ascospore formation is similar to that described for other closely related species. Sexual development differs from that of closely related species principally in the morphology of the ascogonium and in the presence of a trichogyne-spermatium mechanism for diploidization.

Until recently, no thorough cytological study of the development of the perithecium of the oak-wilt fungus had been made. Two preliminary reports (15, 16) were presented in January of 1956 on this subject. Until this time, the nature of the ascogonium, the events leading to fertilization, and the manner of ascospore formation were unknown. The following is a more detailed report of information obtained from a cytological study of the developing perithecium of *Endoconidiophora fagacearum* Bretz.

The first description of the perithecial stage of the oak-wilt fungus, *Chalara quercina* Henry, was made in 1951 by Bretz (5, 6), who renamed the fungus *E. fagacearum*. Bretz obtained perithecia by mating different isolates on agar plates. It was found later by Hepting et al (9) that cultures from single spores fell into 2 groups, now called compatibility types A and B.

Bretz (5, 6) presented a general morphological description of the perithecium of *E. fagacearum*. He recognized that the ascus was of an evanescent nature and considered 8 to be the usual number of ascospores in each ascus. He likened the ascospore's shape and arrangement to the sections of an orange and was unable to find a well-defined ascus wall or membrane. Stessel and Zuckerman (14) studied perithecia found in nature and presented a general morphological description of them. They reported that perithecia devoid of ascospores contained sac-like structures attached by long strands to the inner faces of the perithecial wall. These sac-like structures, which contained 1 or more nuclei, were interpreted as early stages of ascus development. They stated, "Perithecia in a more advanced stage of growth contained globose to sub-globose asci that lay free in the perithecial cavity." These asci were found to contain clearly defined spores arranged in a circular pattern. They were unable to determine definitely the number of ascospores in the ascus.

Cytological studies of perithecial development have been made of species closely related to the oak-wilt fungus. *Endoconidiophora fimbriata* (Ell. & Halst.) Davidson has received considerable cytological attention (2, 7, 8, 12). It is recognized that this species has an unwalled ascus that deliquesces prior to ascospore liberation. There is considerable disagreement on the details of ascospore formation in this species. Other fungi closely related to the oak-wilt fungus that have been studied cytologically include *Endoconidiophora coerulescens* Münch (12), *Endoconidiophora moniliformis* Hedges & Davidson (2), *Endoconidiophora adiposa* (Butl.) Davidson (13), *Ophiostoma multiannulata* Hedges & Davidson (1), and *Ophio-*

[1] Accepted for publication June 28, 1956.

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The author would like to express his appreciation to Dr. H. L. Barnett, under whose direction this work was done.

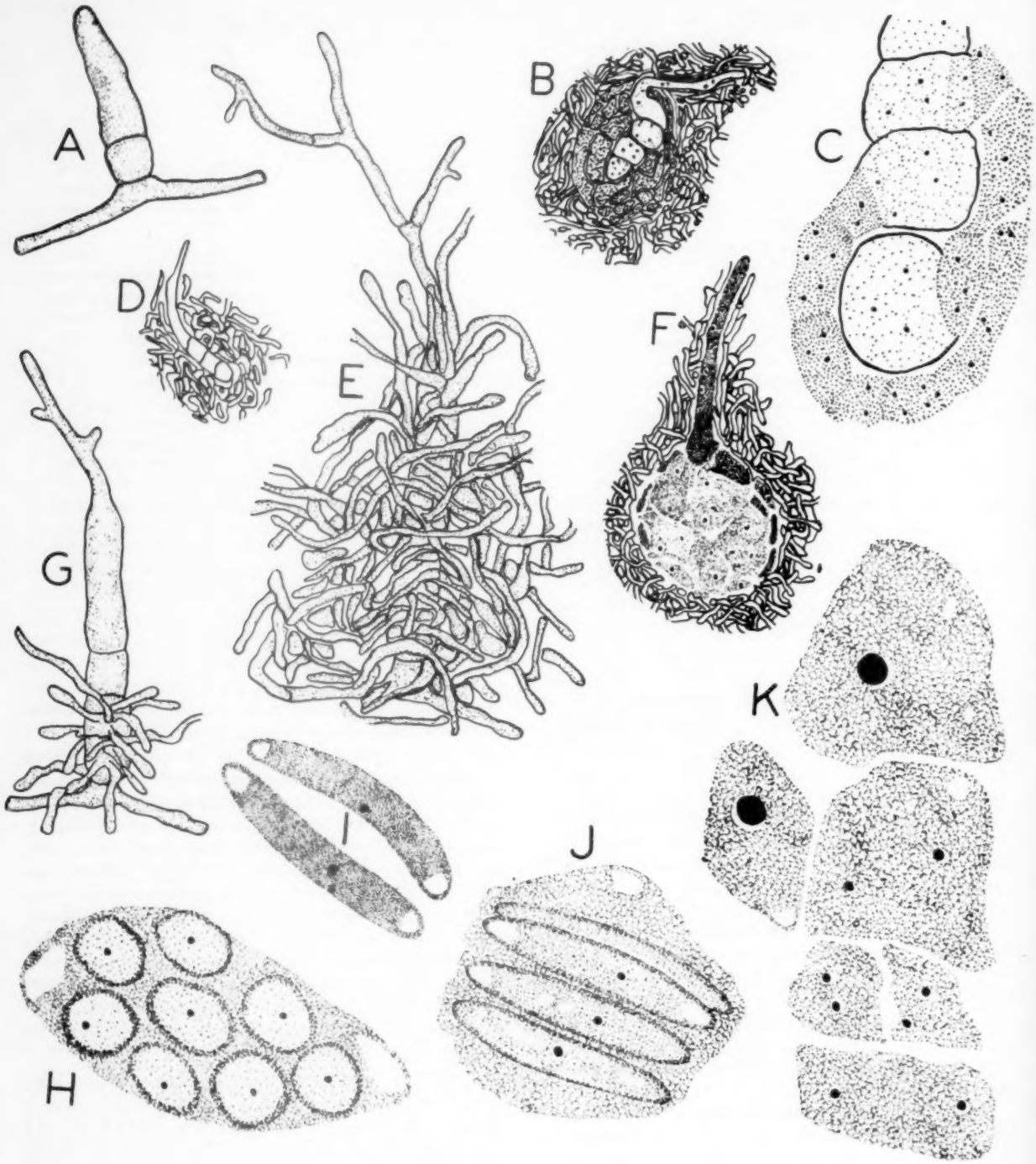

FIG. 1. Drawings of ascogonial and perithecial development. A) Upright, enlarged branch of mycelium (about ×150). B) Perithecial primordium 4 hours after spermatization (about ×37). C) Three basal cells of ascogonium 4 hours after spermatization. Note the chain of cells being cut off by the cell next to the bottom (about ×100). D) Cross section of young ascogonium (about ×30). E) Ascogonium surrounded by sheath of mycelium. Note the trichogyne extending beyond the sheath (about ×150). F) Perithecial primordium 8 hours after spermatization (about ×37). G) Developing ascogonium. Note outgrowths from basal cells (about ×150). H) Ascus containing 8 developing ascospores (about ×570). I) Two uninucleate ascospores. Note terminal vacuoles (about ×570). J) Ascus containing mature ascospores (about ×570). K) Chain of ascogeneous cells and 2 young asci (with large nucleoli). Note paired dikaryotic nuclei of basal cells (about ×570).

stoma majus (van Beyma) Goid. (10).

MATERIALS AND METHODS.—The oak-wilt fungus was grown on the maltose-"Casamino Acids" (Difco) agar medium suggested by Barnett (3). The plates were implanted at 3 points with tufts of mycelium and incubated at 25° C. After a period of 4–6 days, the feltlike areas of the mycelium were spermatized with a conidial suspension of the opposite compatibility type, sprayed on with an atomizer. Material for study was fixed just prior to spermatization, just after spermatization, and at 4-hour intervals thereafter up to 60 hours.

The material was obtained by cutting out small blocks (1–2 cm) of the mycelium and agar. These blocks were taken from raised, feltlike areas of the fungus growth, where perithecial production occurs, and were immediately fixed for 12–24 hours in weak chrom-acetic-acid or formalin-propionic-acid-alcohol (11). The former gave the best results with the stains used.

The blocks were dehydrated in a tertiary butyl alcohol series as recommended by Johansen (11, p. 130) and kept in the mixture of paraffin oil and tertiary butyl alcohol for 2 hours. "Fisher Tissumat" (m. p. 56–58° C) paraffin was used for embedding and proved very satisfactory.

Sections were cut 3–7 μ in thickness and fixed on slides with Mayer's adhesive and Haupt's adhesive (11), with the latter proving far superior. The paraffin was removed with xylol and various stains were used.

Heidenhain's iron haematoxylin, Fleming's triple stain (consisting of safranin, crystal violet and orange G), iron haematoxylin, and safranin were used with acceptable results. The procedures recommended by Johansen (11) for using these stains were followed. Fleming's triple stain, as a rule, gave the best definition of the general structure of the perithecium, but the iron haematoxylin combinations in some cases were superior. The nucleoli were well defined when safranin was used. The use of a green Wratten filter (B 58) improved the definition of some sections under the microscope.

Studies of the early development of the ascogonium were made by examining 3- and 4-day-old cultures growing on agar in Petri dishes. The ascogonia were first formed as naked structures that could be studied by examining living material. It was necessary to use sectioned material for the studies of later development, because sheaths were formed around the ascogonia and dense areas of growth appeared where ascogonia were produced.

CYTOLOGICAL RESULTS.—*Formation of the ascogonium and sheath.*—Ascogonia are first formed as naked structures without surrounding sheaths of hyphae. They are formed prior to the development of feltlike areas of growth and can first be observed as enlarged upright branches of mycelium that differentiate early into a basal cell and an apical elongated cell (Fig. 1, A and 2, A). Upon further differentiation, the ascogonium consists of 3 greatly vacuolated cells and a fourth elongated apical cell (Fig. 1, D, G, and 2, D). The apical cell, which is considered to form the receptive hypha or trichogyne, elongates further and becomes septate and branched (Fig. 1, E). These branches cannot be distinguished from other hyphae except by tracing them to their point of origin.

Hyphalike outgrowths extend from the basal cells of the ascogonium (Fig. 1, G and 2, D). It could not be determined whether these outgrowths originate from the cells of the ascogonium that later produce "ascogenous cells" or from specialized stalk cells at the base of the ascogonium. These outgrowths were observed or recognized only in living material (Fig. 1, G), and they make up part of the ascogonial sheath. In addition, adjacent mycelium becomes associated and grows around the ascogonium to complete the sheath (Fig. 1, E and 2, B). The ascogonium becomes entirely surrounded by mycelium except for the trichogyne, which extends beyond the sheath toward the surface (Fig. 1, E). The ascogonia and the sheaths are formed within a period of 12 hours (Fig. 2, A, B). The formation of the ascogonium and sheath is accompanied by the growth and association of aerial mycelium to form a dense feltlike growth of hyphae that soon obscures the primordia.

Ascogonium at the time of spermatization.—Perithecia were produced in greatest numbers when cultures 4 days old were spermatized on freshly formed feltlike areas of mycelium. The development of the ascogonium can be followed before this time by the examination of living material.

The ascogonium at the time of spermatization consisted of 3 greatly vacuolated, bulbous cells and a trichogyne that extended toward the surface of the mycelium (Fig. 1, D, E and 2, C). It was difficult in sectioned material to follow the trichogyne for any considerable distance because of its hyphal nature, but it was found to occur consistently as a specialized part of the ascogonium. The nuclear condition of the 3 bulbous cells of the ascogonium prior to spermatization could not be determined. The 3 basal cells of the ascogonium may sometimes undergo division before spermatization, but generally this division is not observed until afterward.

Although fusion of a conidium with a receptive hypha was not observed, there is good evidence that this occurs when a conidium comes in contact with a trichogyne of the opposite compatibility type. The nucleus of the conidium is believed to pass through the trichogyne to the ascogonium where it enters into the production of "ascogenous cells." The surface of the feltlike growth above the perithecial primordia at the time of spermatization consists of a dense palisade of conidiophores on which an enormous number of endoconidia are produced (Fig. 2, F). The conidia form a dense crust over the surface of the fungus growth, and it is impossible to distinguish between the spermatizing conidia and those produced by the culture being spermatized.

The ascogenous material of the perithecium is not hyphal in nature. There are no cell walls or localized growing points. The use of the term "ascogenous hyphae" for this material seems unsuitable. Andrus (1) and Hutchinson (10) use the term "ascogenous cells," which seems more descriptive of this mass. They were able to distinguish the early sterile components of the perithecium and did not consider them to be ascogenous cells. In this study, this distinction could not be made and the term ascogenous cells is applied to all cells originating from the ascogonium. The majority of these produce asci, but some differentiate into the sterile components of the perithecium.

Perithecial primordium after spermatization.—About 4 hours after spermatization, a noticeable change takes place in the perithecial primordium. The 3 multinucleate basal cells of the ascogonium divide to produce chains of ascogenous cells (Fig. 1, C and 2, E). These probably are homologous to ascogenous hyphae of most ascomycetes. The ascogenous cells are cut off generally from all 3 ascogonial cells and are multinucleate with no definite pairing of nuclei. There is no definite wall delimiting the ascogenous cells, and they soon disassociate. Each cell then apparently is able to proliferate, producing additional cells. About 8 hours following spermatization,

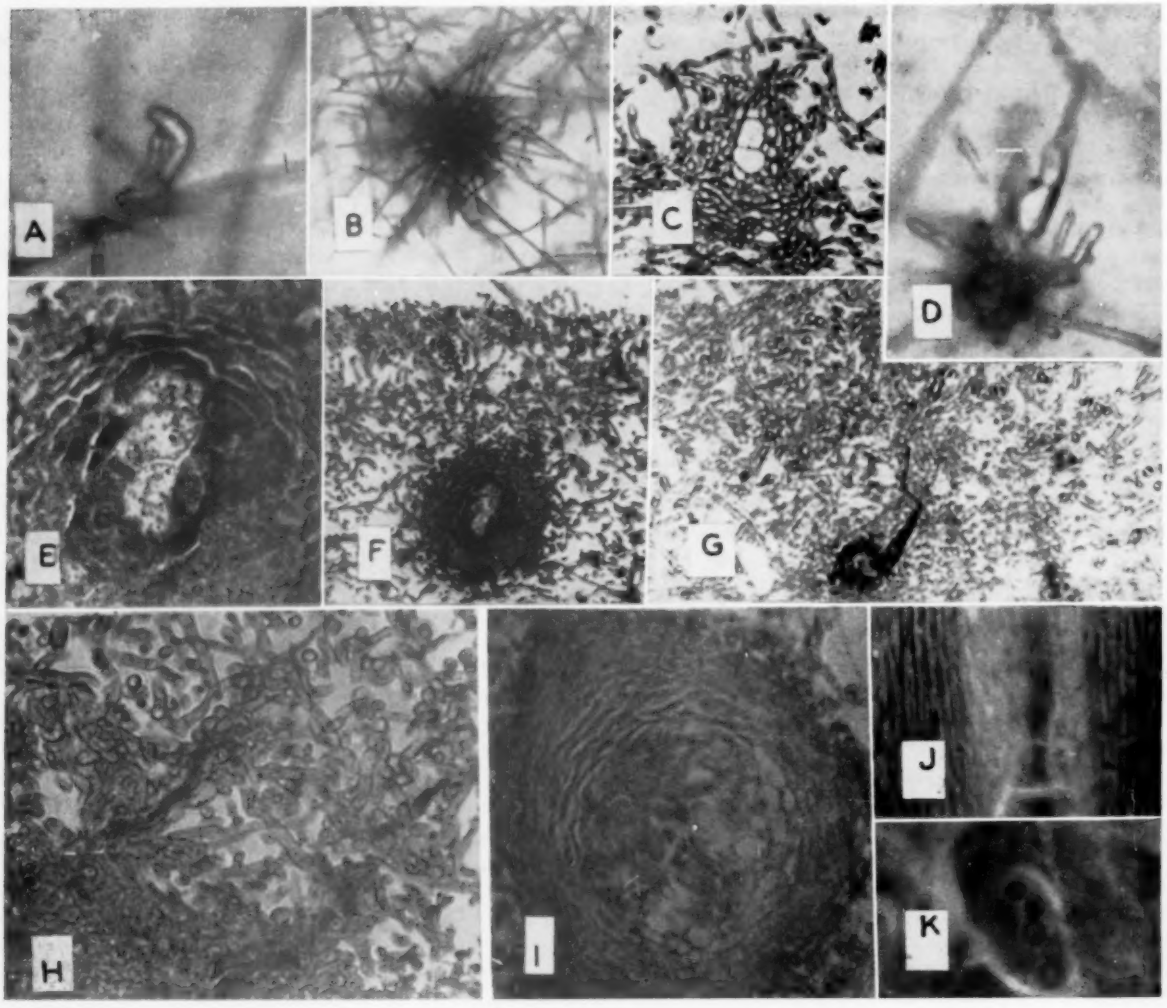

FIG. 2. Photomicrographs of ascogonial and perithecial development. A) Upright enlarged branch of mycelium (about ×60). B) Same ascogonium as A after 12 hours of development. Ascogonium has been surrounded by a sheath of mycelium (about ×30). C) Cross section of young ascogonium and sheath (about ×45). D) Developing ascogonium. Note outgrowths from basal cells (about ×60). E) Three ascogonial cells (light multinucleate cells) cutting off ascogenous cells (about ×50). F) Same as E—showing location in feltlike fungus growth (about ×20). G) Perithecial primordium 8 hours after spermatization. Note trichogyne directed toward the surface (about ×30). H) Two separate trichogynes directed toward the surface 8 hours after spermatization. Note multinucleate condition of trichogyne at left (about ×60). I) Perithecial primordium 20 hours after spermatization. Note initiation of neck formation and differentiation of vacuolated cells (about ×30). J) Logitudinal section of neck. Ascospores (dark-staining) in central canal (about ×70). K) Ascus containing 8 uninucleate ascospores (about ×140).

the 3 basal vacuolated cells of the ascogonium are no longer discernible (Fig. 1, F). They are probably completely utilized in the formation of the ascogenous cells. The basal portion of the trichogyne persists after plasmogamy has occurred and is multinucleate (Fig. 1, F and 2, G). Later it cannot be seen because of its replacement by the rapidly expanding perithecium. The base of the trichogyne is only slightly larger than the hyphae and can be distinguished from them only by its point of origin and its greater affinity for a basic stain (Fig. 1, F and 2, G, H). Although the trichogyne is difficult to follow in sectioned material, it has been observed to extend some distance (105 μ) from the ascogenous cells to the surface of the fungus growth (Fig. 2, G). The nature of the terminal portion of the trichogyne could not be determined in sectioned material, as it seemed to be indistinguishable among the dense mass of hyphae and conidiophores.

The ascogenous cells appear always to lack definite walls, but probably a plasma membrane enables them to maintain their form. The nuclear condition of these early ascogenous cells is difficult to observe because of the density and the deep-staining property of their protoplasm. Nuclei generally appear to be scattered with no definite pairing.

Spermatization is followed by the rapid production of ascogenous cells and the multiplication of nuclei within these cells. After about 20 hours of development, vacuolated cells are distinguishable within the centrum, generally scattered among the ascogenous cells but sometimes concentrated at the base (Fig. 2, I). These cells differentiate from some of the ascogenous cells, and they are considered to be analogous to the "cushion" or "space making" cells described by other authors for other species (8, 10, 13). They probably play a large part in the expansion of the perithecium by the exertion of an internal growth pressure. That the perithecium grows by internal pressure is evidenced by the compression of cells lining the centrum and the cells making up the wall of the perithecium (Fig. 3, D). The breakdown of the large vacuolated cells provides space within the centrum for the formation and liberation of ascospores.

The early formation of the neck of the perithecium can be observed at the time of differentiation of the large vacuolated cells. A greater concentration of the inner cells of the perithecial wall in the neck region is the first apparent step in neck formation (Fig. 2, I). This occurs about 20 hours after spermatization. Neck elongation is not considered to take place by the division of meristematic cells in the neck region, but by the evagination of a portion of the perithecial wall resulting from localized growth pressure exerted within the perithecium. The presence of a large number of cells, continuous with the inner wall of the perithecium, in the area of neck elongation gives the appearance of a region of meristematic activity. This condition probably arises by the accumulation of hyphae in the pathway of neck elongation and a greater compression of hyphae of the inner wall of the perithecium in the region of neck elongation. Vertical hyphal growth in the apical region of the elongating neck also contributes to the formation of the neck (Fig. 3, A).

After about 30 hours of development (Fig. 3, A), the protoplasm of the ascogenous cells is less dense than before and the nuclei are more easily seen. Some nuclei seem to be paired, but it is impossible to distinguish between daughter nuclei and paired conjugate nuclei before fusion. There is no definite wall around the ascogenous cells that, nevertheless, retain their form.

Prior to ascospore formation, the ascogenous cells are arranged in chains with no definite delimitation between cells. These chains are attached to the inner wall of the perithecium and are directed toward the center. The nuclei are distinctly paired at this stage of development (Fig. 1, K and 3, B), and they are believed to represent conjugate nuclei before fusion.

About 40–50 hours after spermatization, the neck of the perithecium has elongated greatly and at maturity consists of an outer layer of laterally compressed hyphae, continuous with the outer wall of the perithecium and an inner layer of delicate, loosely associated cells (Fig. 2, J). Part of the inner layer of cells deliquesces to form the canal of the neck. The cells of the outer layer are closely associated laterally and give rigidity to the neck. The terminal hyphae of the outer portion of the neck form a fringe around the ostiole. The necks of the perithecia average 175–320 $\times$ 42–65 μ.

The wall of the base of the perithecium consists of an outer layer of interwoven and closely associated hyphae. The inner portion of the perithecial wall consists of dark-stained cells that are closely compacted. These cells are long and overlapping, and it is assumed that their condition results from the pressure exerted by the expanding ascogenous cells and vacuolated cells. The diameter of the base of the perithecium varies from 210 to 315 μ.

Some full-sized sterile perithecia with elongated necks were observed 20–60 hours after spermatization. The centrum of such perithecia consisted of large vacuolated cells with no fertile cells present (Fig. 3, C). These are considered to be sterile perithecia that often develop in cultures that have not been spermatized.

Ascospore formation.—The collapse of the vacuolated cells of the centrum creates a space in the center of the perithecium and an opening from the center to the neck canal. The first ascospore formation was observed in the center of the perithecium 40–50 hours after spermatization (Fig. 3, E, F). The ascogenous cells differentiate into smaller, usually binucleate cells, that remain attached in chains. Large vacuolated club-shaped cells like those described by Hutchinson (10) were observed in some cases prior to ascospore

formation.

Karyogamy was first observed in the cells near the center of the perithecium. Nuclear fusion results in a diploid cell distinguishable by a large nucleolus. The young asci are saccate, unwalled, and vacuolated (Fig. 1, K and 3, D). Karyogamy is followed by 3 rapid divisions, which result in an ascus with 8 nuclei (Fig. 1, H and 2, K). The first 2 divisions are probably meiotic and the third mitotic.

Following the nuclear divisions, the protoplasm of the ascus surrounding each of the 8 nuclei rounds up in the formation of 8 ascospores (Fig. 1, H and 2, K). Differentiation of the protoplasm surrounding the nuclei into a denser outer layer can be observed (Fig. 1, H). This outer layer forms the spore wall while the remainder persists as the protoplasm of the spore. Ascospores occasionally were found arranged within the ascus in a circular pattern around a central vacuole in the manner described by Stessel and Zuckerman (14), but generally the ascospores were randomly arranged with no such vacuole present (Fig. 1, H). Not all the protoplasm of the ascus is utilized for ascospore formation (Fig. 1, J). The remaining protoplasm tends to hold the 8 ascospores together in a

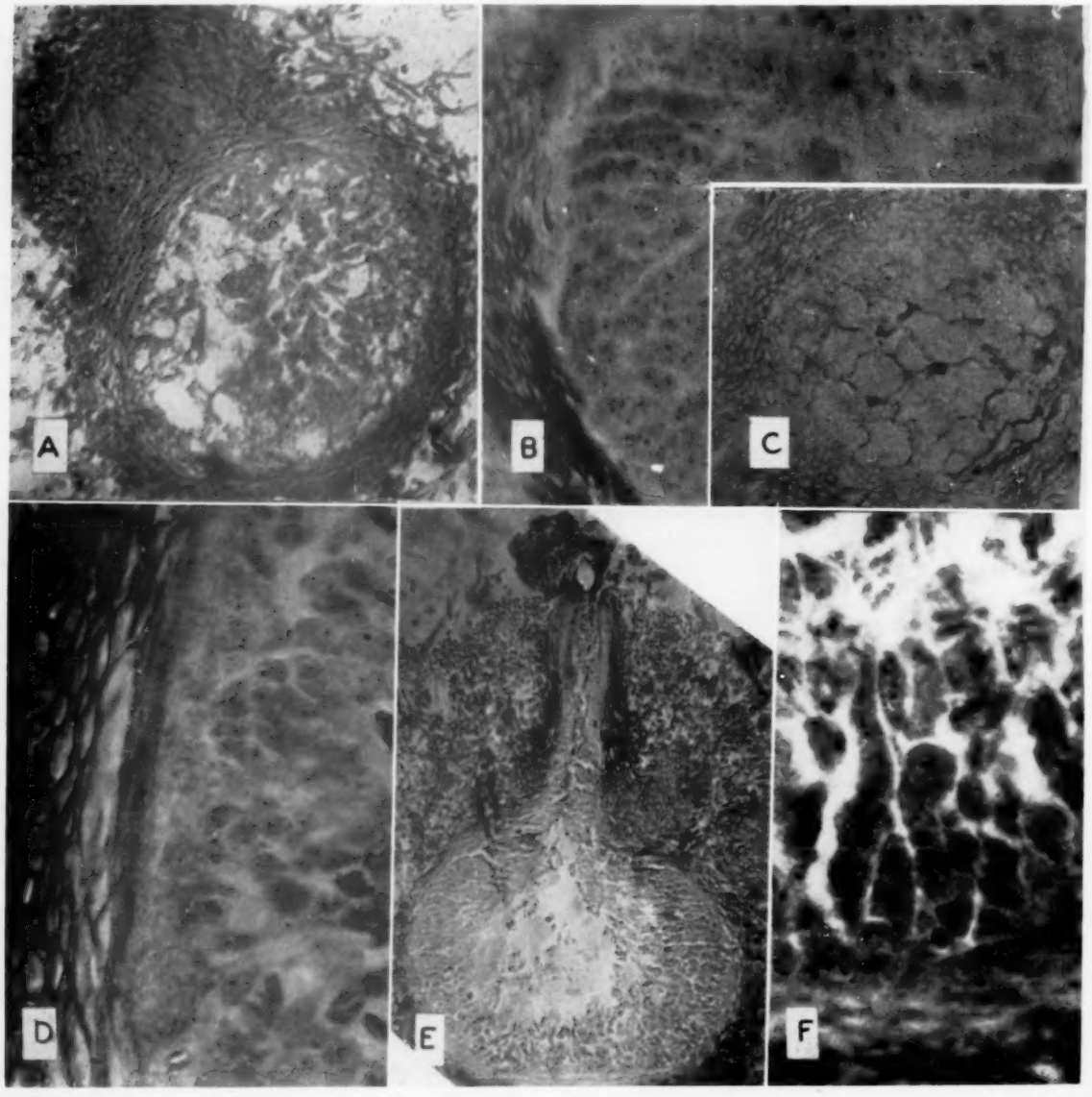

FIG. 3. Photomicrographs of perithecial development. A) Developing perithecium 30 hours after spermatization (about ×30). B) Ascogenous cells. Note paired nuclei (about ×75). C) Sterile perithecium. Centrum consists entirely of vacuolated cells (about ×30). D) Longitudinal section of mature perithecium. Note diploid cells with large nucleoli (about ×150). E) Longitudinal section of mature perithecium (52 hours after spermatization) oozing ascospores (about ×20). F) Mature perithecium—ascogenous cells and asci arranged in chains (about ×150).

packet and may later contribute to the matrix in which they are suspended. A thin layer of the matrix can be seen around each individual spore when ascospores dispersed in water are examined under the microscope. The ascospores average 6–10 × 1.5–3 μ. They are hyaline, slightly curved, uninucleate, and often have terminal vacuoles (Fig. 1, I, J).

Fully formed ascospores are found singly or in groups in the center of the perithecial cavity. The cavity extends from the center of the perithecium to the neck canal. Mature ascospores in a matrix are forced up the neck of the perithecium and are deposited in a moist, sticky ball at the ostiole (Fig. 3, E).

Discussion.—*E. fagacearum* differs from closely related species in that it has an apparently functional trichogyne. Early work by Elliott (7) with *E. fimbriata* indicated that this organism had a trichogyne that made contact with an antheridium to initiate the sexual stage; however, later workers (2, 8, 12) have failed to confirm this interpretation.

The ascogonium of *E. fagacearum* is similar to that of other related species in that it originates from a lateral branch of the mycelium; however, its differentiation into 3 bulbous basal cells and a trichogyne differs from that in other species. The outgrowths from the basal cells of the ascogonium that form part of the sheath are not peculiar to the oak-wilt fungus. This condition was reported by Gwynne-Vaughan and Broadhead (8) for *E. fimbriata* and by Hutchinson (10) for *O. majus*.

Evidence has been presented that demonstrates that nuclear fusion in the oak-wilt fungus involves nuclei from both compatibility types. From Barnett's work (4) on the crossing of albino and dark isolates, it is evident that nuclear fusion is followed by chromosome recombination and segregation of the factors for both pigmentation and compatibility type. In addition, so far as is known, all perithecia produce ascospores of both compatibility types.

Although fusion of a conidium with a trichogyne was not observed, there is good circumstantial evidence that this is the method whereby the genomes of the 2 compatibility types are brought together. All the trichogynes observed were directed toward the surface of the fungus growth, and some were observed to be multinucleate following spermatization. This circumstantial evidence plus the fact that the ascogonia consistently have trichogynes as a specialized part of their structure strongly suggests that the trichogynes normally function as receptive hyphae. The existence of a functional trichogyne does not rule out the possibility that hyphal tips may bring about fertilization of perithecial primordia in cases of intermingling cultures of both compatibility types.

Gwynne-Vaughan and Broadhead (8), Mittmann (12), and Andrus and Harter (2) consider the fertile cells of *E. fimbriata* to originate from a single binucleate cell of the ascogonium. Andrus (1) describes the same condition for *O. multiannulata*. In *E. fagacearum*, all 3 basal cells of the ascogonium function in the production of ascogenous cells; however, the fate of the cells produced from each ascogonial cell could not be followed. The above authors describe the ascogonia of other species as dividing by fragmentation. In *E. fagacearum*, the ascogenous cells are systematically cut off from the 3 basal cells of the ascogonium. Ultimately, the cells of the ascogonium are completely utilized in the formation of ascogenous cells that then independently form more cells.

The development of sterile vacuolated cells within the perithecium is characteristic of the Ophiostoma group. Some workers have emphasized their space-making properties or food storage properties, but no reference has been made to their possible role in the enlargement of the perithecium. The perithecium undergoes much enlargement during the course of its development. This increase in size is accompanied by an enlargement of sterile vacuolated cells. It seems evident that these vacuolated cells, through their absorption of water (which may be due to a conversion of glycogen to sugars), create an internal growth pressure within the perithecium. This pressure would account, in part, for the rapid expansion of the perithecium.

Much variation was found in the growth and multiplication of the ascogenous cells. No crozier formations were noted. Diploid cells are formed by the fusion of conjugate nuclei; fusion occurs first in the center of the perithecium, and progresses toward the perithecial wall until most of the ascogenous material is utilized.

Nothing was observed comparable to a division within an expanded nuclear membrane as described by Andrus and Harter (2). Ascospore formation resembles that described more recently by authors studying closely related species. The nuclear membrane of the diploid nucleus disappears, and the meiotic divisions occur in the cytoplasm of the ascus.

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A PHYTOPATHOLOGICAL NOTE

Ammonium Succinate and Inorganic Ammonia-Producing Materials as Fungicides for the Control of Blue-Green Mold Decay of Citrus Fruits. F. A. GUNTHER, M. J. KOLBEZEN, R. C. BLINN, E. A. STAGGS, J. H. BARKLEY, G. B. WACKER, L. J. KLOTZ, C. N. ROISTACHER, AND A. EL-ANI. Many compounds, including gaseous nitrogen trichloride[1] and biphenyl, have been used commercially to control the blue-green mold fungi (*Penicillium italicum* Wehm. and *P. digitatum* Sacc.) of lemons and oranges during storage and shipment. More recently, Roistacher, Eaks, and Klotz[2] have carefully re-evaluated the utility of gaseous ammonia in this regard. Their data indicate that 2500–5000 ppm of ammonia injected intermittently over a period of 3 days into the free air space inside boxes of lemons and oranges very effectively prevent these molds from developing at inoculated sites of peel wounds, without resulting in fruit surface injury, darkening buttons, or off-flavors in the juice.

As the result of a systematic study of compounds capable of slowly hydrolyzing to release ammonia in a highly humid environment, we have prepared and evaluated over 50 ammonium salts of monocarboxylic, dicarboxylic, and tricarboxylic acids and of half esters of dicarboxylic acids. Among these, ammonium succinate is most promising by virtue of outstanding decay control, probable commercial availability at low cost, favorable toxicology, lack of objectionable odor, hygroscopic nature, and ease of formulation. In Table 1 are shown typical results of tests with several methods of application. For each test, 50 Valencia oranges were injured by the saw method[3] to afford a

TABLE 1.—*Blue-green mold decay and injury of inoculated Valencia oranges treated in various ways with ammonium succinate[a] and then incubated 1 week at 68° F*

Formulation and method of application	Amount used	Percentage infection after 1 week	Injury
Dip[b] (10-20%)		45, 60, 85, 90	
Dry powder sifted on top of fruits in cartons	4-6 g	2	Occasional tiny red spots
Saturated solution poured on paper toweling, top and bottom	10 ml	2.5	None
Powder on bottoms of cartons, covered with paper toweling	6 g	2.5	None
Powder rolled into paper toweling in bottom corners of cartons	6 g	17.5	None
Powder on paper toweling, tops and bottoms of cartons	6 g	0.0	None
Pellets (0.3 g) distributed in cartons	5 g	2.5	None[c]
Pellets (0.3 g) of compound made with ½ weight shredded asbestos, distributed in cartons	5 g	5	None
Slab wax containing 10% ammonium succinate, brushed on fruits before inoculation		58	None
Biphenyl controls[d]	4 g	5, 38, 24, 20	None
Untreated controls		55, 64, 70, 75	

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[3] Roistacher, C. N., and L. J. Klotz. 1955. A device for rapid inoculation of citrus fruits. Phytopathology 45: 517-518.

[a] NH_3 value: theory, 22.4 per cent; found, 22.4 per cent.

[b] Similar results on lemons. It made no difference whether the fruits were dipped before or after inoculation.

[c] One fruit showed wound injury where pellet had actually pressed into inoculation wound.

[d] Two sheets of standard commercially available biphenyl-impregnated liner paper per carton.

TABLE 2.—*Proportional composition and effectiveness of inorganic pellet mixtures[a] for control of blue-green mold decay of inoculated Valencia oranges after incubation for 1 week at 68° F*

Preparation	Proportional composition of pellets					No. of decayed fruit[b]	Injury	Condition of residue at end of test
	NH_4Cl	$(NH_4)_2SO_4$	CaO	MgO	Asbestos			
	g	g	g	g	g			
1	6		3.14		6	1/40	None	Wet paper and pellet
2	6			2.22	6	2/40	None	Moist paper and pellet
3		6	2.54		6	5/40	None	Dry paper and pellet
4		6		1.82	6	0/40	None	Dry paper and pellet
Biphenyl control[c]						12/40	None	Dry paper
Untreated control						35/40		

[a] Six 1-g pellets, individually wrapped in paper toweling, were scattered among the fruits.
[b] Numerator is number of fruti decayed; denominator is number of fruit used.
[c] Two sheets of standard commercially available biphenyl-impregnated liner paper per carton.

wound $1 \times 1 \times 10$ mm, then inoculated with approximately 22,000 mixed spores per wound from a dry spore shower. The fungicide was placed in miniature fibreboard cartons ($6 \times 9 \times 11$ in.) containing the fruits, then the cartons were stored 1 week at 68°F before evaluation of infection and injury.

Large-scale tests of ammonium succinate are in progress. For example, two 10×16-in. liners[4] containing 9 g of powdered ammonium succinate, when placed against opposite walls of a standard-sized ventilated shipping carton containing 150 Valencia oranges injured and inoculated as above, afforded 22 per cent infection (no injury) after 1 week at 68°F. Use of biphenyl liners in parallel tests resulted in 50 per cent infection with some injury; control oranges were 77 per cent infected.

The ammonium salts of adipic, azelaic, caproic, pimelic, sebacic, and suberic acids and the ammonium salts of the acid esters of malonic, succinic, adipic, and sebacic acids also show promise. Effectiveness of these compounds apparently is not correlated with the simple vapor tensions of ammonia over them. Further investigations are under way with these and other organic compounds capable of yielding gaseous ammonia under the conditions existing within a carton of packed citrus fruit.

As the result of a systematic study of compounds capable of releasing ammonia by a chemical reaction induced by absorbed moisture, the easily available ammonia-containing compounds, ammonium chloride or ammonium sulfate, were mixed intimately with weakly basic substances such as calcium oxide and magnesium oxide. All materials were anhydrous and finely powdered in order that they would react to evolve ammonia at a rate proportional to the absorption of water. In addition, shredded asbestos was mixed with the active components and the whole compressed into 1-g pellets.

For each test, 40 Valencia oranges were injured by the saw method, inoculated, and placed in small fibreboard cartons as before. Then 6 g of the ammonia-producing pellets, individually wrapped in small pieces of paper toweling, were scattered among the fruits, the cartons were sealed, and the fruit was incubated for 1 week at 68° F before evaluation of mold control and injury. Table 2 shows the degree of control obtained and the physical conditions of the mold-control materials at the completion of the test.

Further experiments are underway in which the anhydrous reactants are contained in powder form in paper mats[5] used to line the cartons. Also under investigation are the effects of various factors on the rate and duration of evolution of ammonia, such as the size of pellets, the presence in the pellets of exfoliants such as bentonite, and the addition of dehydrating agents such as magnesium sulfate.—Departments of Entomology and Plant Pathology, Citrus Experiment Station, University of California, Riverside, California.

[4] The compound was spread on a sheet of polyethylene laminate paper and covered with a sheet of wrapping paper, then the 2 sheets were sealed in a waffle pattern with a hot soldering iron.

[5] The mixtures were spread on a sheet of absorbent porous paper, covered with a sheet of polyethylene laminated paper, and the 2 sheets sealed by heat in a waffle pattern. The porous sheet was faced inward toward the fruit.

REPORT AND ABSTRACTS OF THE THIRTY-EIGHTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The thirty-eighth annual meeting of the Pacific Division of the American Phytopathological Society was held on the campus of the University of Washington, Seattle, Washington, June 13 to 15, 1956, in conjunction with the annual meeting of the Pacific Division of the A. A. A. S. Included on the program were 44 papers and a field trip to the Puyallup Valley and the Western Washington Experiment Station at Puyallup. The Phytopathologists' dinner on Thursday evening, June 14, was attended by 90 members and guests. Dr. William Newton served as Master of Ceremonies.

The membership voted unanimously to forego a division meeting in 1957 and instead to meet jointly with the parent society at Stanford.

Officers elected for the year 1956-57 were: *President*, Roy A. Young; *Vice-President*, C. S. Holton; *Secretary-Treasurer*, W. A. Kreutzer; and *Councilor*, Roderick Sprague.

ROY A. YOUNG, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Strains of the barley yellow-dwarf virus. ALLEN, THOMAS C., JR. Research concerning strains of the barley yellow-dwarf virus was instigated in January of 1954. Following the inoculation of 31 cereal varieties with each virus isolate, symptoms on any one variety differed with different isolates. Four of the 31 cereal varieties were then selected as differential hosts for testing all isolates. The varieties chosen were the barley varieties Black Hulless (C.I. 666), Rojo (C.I. 5401), and Atlas 46 (C.I. 7323), and the oat variety Coast Black (C.I. 1025). Forty-three yellow-dwarf isolates were obtained from various states of the United States. When transferred to the selected hosts, 16 strains were distinguished. The separation was based on virulence of the virus on the 4 hosts, and was statistically significant at the 1 per cent level. All isolates were tested on the differential hosts at least twice and some up to 8 times. The strains could be arranged in 7 groups based on their ability or inability to incite symptoms in each host. Two of the types thus separated included 10 of the 16 strains.

Comparison of initial kills and subsequent increase of nematode populations following soil fumigation. ANDERSON, E. J. Results from application of D-D mixture (1,3-dichloropropene; 1,2-dichloropropane), ethylene dibromide, and 1,2-dibromo-3-chloropropane at 3 soil moisture levels, with and without paper covers, at 2 dosage levels each in field plots indicate different activities for the 3 materials and confirm the report of Ichikawa, Gilpatrick, and McBeth on 1,2-dibromo-3-chloropropane. The nematode observed was *Rotylenchulus reniformis*. The soil was a low humic latasol. The best initial kills of nematodes were obtained by the use of D-D mixture at 40 gal. per acre applied as a row treatment under cover, a confirmation of earlier reports. The poorest initial kills were by 1,2-dibromo-3-chloropropane, which gave only slightly poorer kill than did ethylene dibromide. Eight months after fumigation, during which time pineapple plants were grown on the plots, sampling for plant parasitic nematode populations showed that the increase of populations had been greatest in plots treated with ethylene dibromide and least in those treated with 1,2-dibromo-3-chloropropane. After 8 months, there was no good evidence of interaction of fumigant or dosage with soil moisture or method of application except for ethylene dibromide, which was more effective at higher moisture levels.

Action of certain antibiotics on downy mildew of cucumber. ARK, PETER A., AND JAMES P. THOMPSON. Downy mildew of cucumber, caused by *Pseudoperonospora cubensis*, was successfully controlled under greenhouse conditions by endomycin (500 ppm), gramicidin S (500 ppm), patulin (50 and 100 ppm), and streptomycin (100 and 200 ppm). Both patulin at 25 ppm and endomycin at 250 ppm gave from 85 to 90 per cent control of the disease. Terramycin, at 100, 200, and 500 ppm, gave 70, 75, and 75 per cent control, respectively, whereas polymyxin and mycostatin, at 100 and 500 ppm, respectively, showed 70 and 90 per cent control, respectively. No protection from downy mildew was obtained by the use of the following antibiotics: aureomycin, 100 and 200 ppm; bacitracin, 100 and 200 ppm; Chloromycetin, 100 and 200 ppm; gramicidin (American), 500 ppm; griseofulvin, 440 and 880 ppm; neomycin, 100 and 200 ppm; penicillin G, 500 ppm; and tetracycline, 100 ppm. Very slight injury on the leaves was observed from patulin, 100 ppm. All materials were used as sprays, and the pathogen was applied 15 minutes after the spraying.

Movement of streptomycin into plant tissues from streptomycin-pyrophyllite formulations. ARK, PETER A., AND EUGENE M. WILSON. The juice of the primary leaves of bean plants dusted with streptomycin-pyrophyllite formulations containing 500 and 1000 ppm streptomycin showed 0 ppm and 7 ppm streptomycin, respectively, when bioassayed after 1 day, and 6 ppm and 5.5 ppm after 3 days. The trifoliate leaves contained 0 and 5.5 ppm, respectively, after 1 day and no detectable streptomycin 3 and 4 days after the dusting. Dipotassium phosphate or glycerol in the dust did not improve the penetration of the antibiotic into bean leaves and the combination of both definitely prevented penetration. The juice from cucumber leaves receiving 1000-ppm dust, alone and plus 1 per cent glycerol, showed 7 ppm streptomycin after 1 and 6 days, whereas with 1 per cent glycerol plus 1 per cent K_2HPO_4, bioassay revealed no streptomycin absorption. In another set of experiments, cucumber leaves dusted with streptomycin pyrophyllite formulations containing 500 and 1000 ppm streptomycin showed 6 and 6.5 ppm streptomycin, respectively, after 1 day, 3 and 5.5 ppm, respectively, after 3 days, and none in the newly developed leaves that appeared 4 days after the dusting. Thus there appears to be a differential penetration of streptomycin into the tissues of different plant species from different streptomycin dust formulations.

Hot water treatment of apple stocks for the control of nematodes. BOSHER, J. E., AND W. NEWTON. Endoparasitic nematodes of the genus *Pratylenchus*, chiefly *P. penetrans*,

were found in approximately 75 per cent of clonal apple stocks recently imported and growing in nurseries in British Columbia. Populations in general were small, the highest count being 235 specimens of Pratylenchus per g of feeder roots. Root injury was usually associated with populations of 80 per g or over. The nematodes in small maiden stocks of East Malling VII and IX were destroyed by immersion of the roots in water at 110°F for 60 minutes, 112°F for 40 minutes, 115°F for 20 minutes, 118°F for 15 minutes, 121°F for 7 minutes, 124°F for 2½ minutes, or 127°F for 1 minute. Larger stocks required longer immersion periods. Treatments were more effective when stocks were soaked prior to treatment for 2 hours in water at 70°F. In these experiments, the plants were cooled in running tap water immediately after the heat treatment. The least injury by treatments occurred when the plants were taken from cold storage at 34°–38°F, treated in January, and returned to cold storage until spring planting. A 1-minute dip in 0.5 per cent Tersan (75 per cent thiram) following treatment appeared to protect the roots from secondary invasion by fungi.

Prune dwarf virus and the sour cherry viruses. CAMERON, H. R. AND J. D. MOORE. Eighty-five lots of Prunus budwood were indexed in an attempt to find the prune dwarf virus alone and in combinations with sour cherry viruses. The budwood was collected from various locations in the United States and Canada on the basis of its reported virus content. The viruses that cause green ring mottle, bark splitting of Montmorency, reactions on Shiro-fugen and Kwanzan, or symptoms on cucumber were found independently or together in various lots of budwood containing the prune dwarf virus. No relationship was found between any of these viruses and the prune dwarf virus. Two lots of budwood that carried the prune dwarf virus did not contain the viruses causing necrotic ringspot and sour cherry yellows. Numerous lots of budwood contained the viruses causing prune dwarf, ringspot, and yellows. Some budwood carried the viruses inciting ringspot and yellows without the prune dwarf virus. Others contained the ringspot virus without those causing yellows or prune dwarf. All possible virus combinations except those that would require yellows to be separated from ringspot were found. Therefore, the prune dwarf virus appears to be a distinct virus from the viruses causing ringspot and yellows and to be capable of inciting symptoms independently.

Control of plant diseases by soil-surface treatment. CAMPBELL, LEO. Experiments at the Western Washington Experiment Station indicate the promise of soil-surface treatment in plant disease control and that this method of treatment has been too often neglected in the past. Numerous experiments have shown that mint rust may be effectively controlled by a pre-emergence soil-surface spray of dinitro amine or of certain fungicides. Pentachloronitrobenzene sprayed on the surface of the soil immediately after seeding reduced Corticium black root of cauliflower as much as 97 per cent. Early postemergence sprays of dichlone and of pentachloronitrobenzene reduced Sclerotinia stem rot of beans 50 and 37 per cent, respectively. A preharvest dust of captan following each cultivation reduced Botrytis fruit rot of strawberries 75 per cent. These results indicate that early soil-surface treatments aimed at preventing the increase of inoculum may prove to be a safe, effective, and economical way to control many other diseases of the above-ground parts of plants.

Prune dwarf expression in plum varieties. COCHRAN, L. C. Prune dwarf has been described as a disease of Italian Prune and Lombard plum; it also causes stunting in peach. Natural occurrence, with symptom expression on 1 Tragedy and 3 Standard prune trees near Beaumont, California, suggested that symptom expression was not limited to Italian Prune and peach. Experimental inoculation has produced prune dwarf symptoms on Italian Prune, Giant, Standard, Emilie, President, and Tragedy (all *Prunus domestica* L.) but no symptoms on Becky Smith, Red June, Abundance, Burbank, Flaming Delicious, Santa Rosa, Late Santa Rosa, Beauty, Wickson, Eldorado, Mariposa, Methley, Green Gage, Elephant Heart, and Apex (all *P. salicina* Lindl.). Tragedy prune was the most severely affected and should prove to be a better test plant than Italian Prune.

Effectiveness of preplanting treatments with antibiotics in preventing crown gall on Mazzard cherry. DEEP, IRA W. In 1954 and 1955, experiments were conducted to evaluate the effectiveness of preplanting treatments with Agri-mycin (15 per cent streptomycin; 1.5 per cent oxytetracycline), streptomycin, and terramycin (oxytetracycline) in preventing infection of Mazzard cherry trees by *Agrobacterium tumefaciens* and to determine whether these materials would act chemotherapeutically against incipient infection. One-year-old trees were root pruned, inoculated by dipping in a suspension of the crown gall bacterium, and treated for 1 minute, 15 minutes, 1 hour, 2 hours, or 3 hours in solutions containing 100, 200, or 400 ppm of each antibiotic. Treatment for 1 hour or longer in 400 ppm of terramycin was most effective against crown gall but was also phytotoxic. The most effective treatments with no resultant phytotoxicity were 15 minutes in 400 ppm terramycin or 1 hour in 200 ppm of terramycin. The incidence of gall following these treatments was 49 and 41 per cent, respectively, as compared to 99 per cent in the inoculated control. In 1 trial, the incidence of crown gall in trees treated in 400 ppm of Agri-mycin for 1 hour was 5 per cent as compared to 20 per cent in the noninoculated control. This significant reduction in the incidence of gall indicates chemotherapeutic action against incipient infections.

Developmental cycles and nuclear phenomena of Ustilago spegazzinii var. agrestis in culture. DIETZ, SHERL M. Teliospores of *Ustilago spegazzinii* var. *agrestis* germinate in approximately 20 hours at the optimal temperature of 30°C. These spores possess bipolar areas functioning as germ pores through one of which the promycelium emerges. If germination occurs through both caps, only 1 promycelium is functional. This elongated promycelium produces lateral hyphae that eventually develop into extensive mycelium upon which are borne aerial sporidiophores with short branched and unbranched chains of sporidia. The terminal sporidium forms a minute projection, from the end of which a swelling arises and gradually enlarges. When this swelling is about half the size of the sporidium from which it is produced, nuclear division takes place and 1 daughter nucleus migrates into the newly formed sporidium. This repetitive process forms short chains. Sporidia of the same sporidiophore will not fuse but generally germinate from both ends and form a ramifying mycelium from which aerial sporidia again arise, constituting a secondary sporidial cycle. Fusions were observed on water agar between sporidia obtained from sporidiophores from different areas of teliospore cultures. Hyphal anastomoses were observed. These apparently give rise to dicaryotic infection hyphae. Giemsa stain was employed in the nuclear investigations.

Soil pasteurization with high frequency energy. EGLITIS, MAKSIS, FOLKE JOHNSON, AND EDWARD P. BREAKEY, JR. In studies on the effect of high frequency energy on the control of seedling damping-off, soil samples highly contaminated with *Pythium* sp. were used. Two types of machines were employed. Generator I was a locally assembled laboratory model with power of about 0.75 kw. Generator II was a commercial Mann-Russell unit, model 200, with power of 5 kw. Both were operated on a frequency of 27 MC. With Generator I, the soil was held in cylindric ice cream cartons with a volume either of 295

or 858 cc. Separate samples were held between brass electrodes (diameter, 15 cm) for varying periods of time. In most instances, damping-off was controlled after a 5-minute exposure to the radio waves. With Generator II, the soil was held in wooden greenhouse-flats, which were then placed directly between the aluminum electrodes. Output of the electronic tubes was 1 amp., and the test plant was crimson clover, of which 750 seed with germination of 90 per cent were sown in each flat after the soil treatment. No damping-off resulted in the flats with Sultan soil exposed 5 minutes or longer to the energy. The seedling stand was 85.5 per cent of the expected, whereas in the check the stand was 24.4 per cent. With Puyallup silty loam, after a 5-minute exposure, the stand was 84.7 per cent and in the check, 9.1 per cent.

Effect of Vapam as a soil fungicide in control of Phytophthora root rot of avocado trees. ERSPAMER, J. L., AND G. A. ZENTMYER. Several concentrations and methods of application of Vapam (sodium methyldithiocarbamate) were used on avocado trees infected with *Phytophthora cinnamomi*. Infection was reduced at 50 to 100 ppm and eliminated in some plots at 150 to 200 ppm. Damage to living roots was severe at the higher concentrations. The allowable borderline appeared to be 100 ppm. Solutions were applied in basins and by sprinkler. With sprinklers, the material was applied in concentrated solution followed by water, or as a solution of uniform strength. Field data indicate that the effective use of Vapam is conditioned by the stage of the disease, type of soil, weather at time of application, solution strength, gallons applied per sq. ft., and method of application. Soil-tube columns were set up in the laboratory to determine conditions whereby 50- and 75-ppm solutions penetrate the soil at killing strengths to 24 in. Variables were soil type, solution strength, gallons applied per sq. ft., soil moisture, and pH. Inoculum was placed at 6-in. intervals at depths from 6 in. to 24 in.; soil moisture and pH at these depths were determined before and following treatment. Data suggest that special attention should be given to the number of gallons applied per sq. ft. and to use of water following application of solutions.

Comparative determinations by electrophoresis, electron microscopy, and local-lesion assay of the growth curve of tobacco mosaic virus in directly inoculated leaves of Nicotiana tabacum. GOODCHILD, D. J., AND MORRIS COHEN. Six expanded leaves on each of 91 greenhouse-grown plants of *N. tabacum* were inoculated with TMV, 10^{-2} mg per ml. In each of 13 subsequent samplings over a period of 30 days, 35 leaves, selected in a predetermined arrangement, were removed. These were homogenized in 0.5 M maleate buffer, pH 7.5, and centrifuged anaerobically. The resulting light yellow whole cytoplasm was subjected to electrophoretic analysis, from which a quantitative determination of the virus content was made. The virus rods present in this whole cytoplasm were counted by means of electron microscopy and the biological activity of the preparation was determined by local-lesion assay on *N. glutinosa*. Biological activity was measurable over the entire period of infection, but virus was not detected electrophoretically until 5 days after inoculation. The necessity for diluting whole cytoplasmic proteins to render virus particles visible by electron microscopy precluded any accurate particle counts during the first 2 days of infection. The curves corresponding to the 3 methods of assay paralleled each other, suggesting that the specific activity of the virus remains constant during its course of synthesis. The minimum generation time of 8 hours occurred between the second and fourth days of infection.

The use of tile for studies on soil-borne pathogens. GOULD, CHARLES J. Investigations of certain soil-borne pathogens of ornamental crops have been hampered by the lack of uniform infestation in fields in western Washington. This has been overcome for preliminary studies on *Sclerotium rolfsii*, *Stromatinia gladioli*, and certain other fungi by the use of soil artificially infested with oat cultures contained in 36-in. belled sewer tiles with an inside diameter of 30 in. These were embedded 18 in. in field soil and surrounded by a 6-in. layer of sawdust to reduce weed growth and splashing of water. A sandy subsoil was placed in the bottom, and the desired soil type added on top to the level of the outside soil. Oat cultures, fungicides, etc., were incorporated with an electric mixer. When bulbs were to be planted, treated soil was removed to the depth desired and placed temporarily in formalin-disinfested cans. Four replications were used in the first experiment, but the results were so uniform that 3 replications have been used in subsequent studies. The latter have included longevity studies of pathogens in different soil types, effects of fertilizers on disease development, and studies of fungicides involving methods of application, kinds, rates, persistence, and effect of soil types. Plant growth has been normal.

Some factors affecting germinability of teliospores of Ustilago tritici before and after lyophilization. GRAHAM, S. O. The lyophile procedures of Raper and Alexander were used in investigations conducted to determine when lethal effects occurred during the various phases of their process. Dry teliospores in various suspensions were quick-frozen, lyophilized without previous quick freezing, and quick-frozen and then lyophilized. Germination rates were plotted against time and compared with untreated checks. After quick freezing, dry teliospore samples germinated only slightly less than checks; samples in suspensions were greatly reduced in germinability, although the higher the colloidal content of the suspending medium, the lower the mortality of spores. Samples lyophilized without previous quick freezing followed the same pattern, except that mortality was considerably less in samples in suspensions. Samples in suspensions quick-frozen and lyophilized had very low germinability levels, indicating 2 distinct losses, whereas dry teliospores were affected no more by quick freezing followed by lyophilization than by quick freezing alone. Lyophilized samples with or without previous quick freezing showed marked increases in germinability if conditioned in a water-saturated atmosphere 24 hours prior to plating out, an indication of a loss of germinability in the reconstitution phase of lyophilized spores. Lyophilized dry spores with or without previous quick freezing had germination levels comparable to those of untreated checks after such conditioning.

Factors for pathogenicity in Ustilago avenae. HALISKY, P. M., AND C. S. HOLTON. The genetics of pathogenicity was studied in crosses between races A5, A6, and A7 of *Ustilago avenae* (Pers.) Rostr. The homozygosity for pathogenicity of each race was first established by testing inbred lines on 6 host varieties. Sporidial combinations were then made representing the following race crosses: A5 $\times$ A6; A5 $\times$ A7; and A6 $\times$ A7. Hybrid spores representing the F_1 progeny from these crosses were selected for a study of segregation for pathogenicity in the F_2. Four inbred lines from 1 hybrid spore constituted an F_2 population. Certain F_2 populations from all of these crosses expressed a 3:1 ratio of factors segregating for pathogenicity. In the crosses of A6 $\times$ A7, the F_1 line suggested simple recessive inheritance of factors for the A7 pathogenicity. All the F_2 populations from this cross expressed 3:1 segregating ratios for pathogenicity factors. Spores from each of the 4 inbred lines of one F_2 population were selected for further inoculation studies. Both the F_3 and F_4 populations of this cross indicated a 1:2:1 segregation of pathogenicity factors. This confirmed the 3:1 ratios indicated by the F_2 data. On the basis of this evidence, it is suggested that in certain crosses between races of *Ustilago avenae* pathogenicity is governed by 1 recessive factor pair.

The perfect stage of Coryneum cardinale. HANSEN, H. N. Since the Monterey cypress canker was first reported in

California in the late twenties, this disease has spread with almost epidemic rapidity. This was difficult to explain, as the causal fungus, *Coryneum cardinale* Wagener, is not ordinarily wind borne. Early in 1954, a wind-borne stage was found in the form of mature perithecia of a *Leptosphaeria* sp. in cankers on a cypress branch that had been dead for at least 2 years. Single-ascospore cultures of this fungus produced typical colonies of *C. cardinale*. The perfect stage was produced *in vitro* on gas-sterilized cypress twigs inoculated with many ascospores, or with conidia from several single-ascospore cultures, but never from a single-ascospore culture, indicating that the fungus is heterothallic. Also observed, both *in vivo* and *in vitro*, was a third spore form consisting of nonviable filiform stylospores, presumably spermatia, borne in structures intermediate between pycnidia and acervulae. Since the first discovery of *Leptosphaeria* sp., it has been found on dying and dead cypress trees over a large area of north central California.

The perfect stage of Pestalotia. HANSEN, H. N., GEORGE A. ZENTMYER, AND W. C. SNYDER. The genus *Pestalotia*, described in 1939, has been known widely for its cosmopolitan imperfect saprophytes, especially on dead or dying leaves and twigs. Recently, an isolate from necrotic areas in an avocado leaf produced perithecia of Leptosphaeria in pure culture. Single-ascospore cultures have yielded the conidial stage and later, in the same cultures, mature perithecia, indicating homothallism. Development of perithecia has occurred only on specialized natural media, such as rusted rose leaves, gas-sterilized and imbedded in water agar. Two out of 12 isolates of Pestalotia obtained from various substrates in nature have developed perithecia on this rusted-rose-leaf medium but not on healthy-rose-leaf medium.

Variation in sensitivity to atmospheric fluorides among Ponderosa pine seedlings. HENDRIX J. WALTER. Observations of several thousand trees and seedlings of *Pinus ponderosa* growing on numerous sites within a fluoride pollution area near Spokane, Washington, revealed a wide range in sensitivity to this atmospheric pollutant. Differences in the incidence and severity of needle browning between individual plants were observed. Critical measurements of foliar samples collected from approximately 1000 even-aged seedlings, growing on sites representing ¼-, ½-, 1-, and 2½-mile distances from the pollution source, further revealed a variation of from 0 to 100 per cent in the number of needles burned and a variation of from 0 to 86 per cent of the total needle length burned on individual plants. During the 4-year period 1951-1954, inclusive, some plants consistently manifested a high degree of sensitivity whereas others remained symptomless. Certain other plants, however, fluctuated in reaction, becoming severely burned one year but showing no such tendency the next. Since "resistant" and "sensitive" plants often existed side by side, sometimes as close as 2 in., exposure differences could not be used to explain the variation. Neither was it explainable, based upon 2 years' experimentation, on a basis of soil moisture, soil fertility, or date of bud elongation.

Nematode control versus application depths of Nemagon. ICHIKAWA, S. T. The effect of placement depth on the activity of Nemagon was studied by injecting this nematocide into a field of sandy loam heavily infested with root knot nematode, *Meloidogyne incognita* var. *acrita*. Nematode control was measured by growing an indicator crop both in the treated plots and in soil samples taken from the plots. Liquid Nemagon injected 2 in. deep with a hand gun gave almost complete control in the surface 12 in. Deeper injections penetrated deeper, but 10- and 12-in. injections failed to kill a few nematodes near the surface. At application depths of 4–8 in., Nemagon impregnated on Attaclay gave as good control in the upper 24 in. of soil as did the liquid. In the 24–36-in. zone, the liquid was slightly more effective than the granules. For most purposes, a 6- to 8-in. placement of Nemagon is recommended for both good penetration and surface control.

A decline of peppermint caused by an ectoparasitic nematode, Longidorus sylphus. JENSEN, HAROLD J., AND C. E. HORNER. A decline of commercial peppermint (*Mentha piperita* var. *Metcham*) has been observed as areas of poor growth in some Oregon plantings since 1953. The surviving plants in affected areas remain stunted and usually exhibit a reddish color. The root systems are poorly developed, with most of the feeder roots reduced to short stubby remnants. Nematodes (*Longidorus sylphus* Thorne) frequently are congregated on the roots of diseased plants in such large numbers that they resemble small tufts of cotton. Pathogenicity of this pest was demonstrated by obtaining field infested soil and comparing the growth of peppermint in flats of steam-sterilized soil and of nonsterilized soil. The most striking differences occurred when rhizomes were used as planting stock, although rooted cuttings also were severely injured. Preliminary soil fumigation experiments are encouraging, but this method of control has not been perfected to a point where general recommendations can be made.

Fungi associated with Oregon-grown clover seed. LEACH, CHARLES M. Examination of 78 samples of seed of *Trifolium incarnatum*, *T. pratense*, *T. repens*, and *T. hybridum*, by a malt agar plating method, revealed that 6 to 47 per cent of the samples were infested with the following potentially pathogenic fungi: *Ascochyta imperfecta*, *Botrytis cinerea*, *Fusarium roseum*, *F. oxysporum*, *Phoma trifolii*, *Sclerotinia* sp., *Stremphylium botryosum*, *S. sarcinaeforme*, and *Verticillium* sp. Additional micro-organisms contaminating the seed were *Alternaria tenuis*, *Aspergillus fumigatus*, *A. niger*, *A. ochraceous*, *Cephalosporium* sp., *Cephalothecium* sp., *Chaetomium cochliodes*, *C. funicola*, *Cladosporium cladosporioides*, *C. elatum*, *C. macrocarpum*, *Didymium difforme*, *Epicoccum* sp., a mucoraceous species, *Nigospora cryzae*, *Penicillium javanticum*, *P. novae-zeelandiae*, *Penicillium* spp., *Pullularia* sp., *Rhizopus* sp., *Scopulariopsis brevicaulis*, *Stemphylium* sp., *S. consortiale*, *Trichoderma* sp., a yellow bacterium, and a white bacterium. In greenhouse pathogenicity tests with artificially inoculated seeds, *Ascochyta imperfecta* caused a 79 per cent loss of seedlings; *Botrytis cinerea*, 95 per cent; *Fusarium roseum*, 62 per cent; *Stemphylium botryosum*, 47 per cent; and *Phoma trifolii*, 85 per cent. The spores or mycelia of most of these fungi were loosely attached to the seed coats, or mycelia were present within the seed coats.

The effects of carbon tetrachloride, air, and water at 158°F on the microflora and germination of clover seed. LEACH, CHARLES M. Seeds of *Trifolium incarnatum* L. were heat treated in carbon tetrachloride, water, and air at 158°F for periods up to 84 hours. In CCl_4 there was no reduction of germination even after the maximum steep of 84 hours. After 1 hour, however, the fungus population (*Cladosporium cladosporioides*, *Phoma trifolii*, *Penicillium* sp., *Alternaria tenuis*, *Stemphylium botryosum*, *S. consortiale*, *Fusarium roseum*, *Sclerotinia* sp., and *Epicoccum* sp.) decreased by 65 per cent, whereas the 84-hour treatment caused a 93 per cent reduction. The potentially pathogenic fungi, *P. trifolii*, *S. botryosum* and *F. roseum*, were eliminated from the seed after 4, 4, and 12 hours, respectively. The *Sclerotinia* sp. was unaffected even after 84 hours in CCl_4. In air, a 1-hour treatment caused a 15 per cent reduction of germination and a 36-hour treatment killed the seeds. The fungus microflora was reduced 50 per cent by the 1-hour treatment in air and completely killed after 8 hours. In water, no seeds survived the 1-hour treatment and the fungi were reduced 95 per cent. It is suggested that the seed germination was not affected in CCl_4 at 158°F because of the exclusion of oxygen. CCl_4 failed to

show any fungicidal effect at room temperature, indicating that the decrease in fungus infestation in this medium at 158°F was mainly a thermal effect.

Effect of some phenols and quinones on growth in culture of Verticillium albo-atrum. LeTourneau, Duane, John G. McLean, and James W. Guthrie. Field experiments have indicated that foliar applications of certain phenolic compounds may alter the resistance of potatoes to *V. albo-atrum*. The effect of molecular arrangement was studied in culture by adding phenolic or quinone compounds to a sucrose-salt medium with 10^{-3}M catechol (*O*-dihydroxybenzene) as a standard. At this concentration, resorcinol (*m*-dihydroxybenzene) was without effect; hydroquinone (*p*-dihydroxybenzene) was intermediate. Replacement of 1 hydroxyl of catechol with a carboxyl (salicylic acid) decreased toxicity; replacement of both hydroxyls (phthalic acid) gave no inhibition. Growth was not inhibited at 10^{-3}M of 2,5-, 2,4-, or 3,4-dihydroxybenzoic acid (protocatechuic) whereas 3,4-dihydroxybenzaldehyde was less effective than catechol. Phloroglucinol (1,3,5-trihydroxybenzene) did not affect growth, but the 2 isomers gave complete inhibition. The quinones were more inhibitory than phenols. Fifty per cent inhibition was found with 2.2×10^{-4}M *p*-benzoquinone, 10^{-4}M tetrachloro-1,4-benzoquinone (chloranil), 5×10^{-6}M 1,4-napthoquinone, and 10^{-6}M 2,3-dichloro-1,4-napthoquinone (dichlone).

Survey for soils in Oregon free of root-lesion nematodes. Loring, L. B., and Harold J. Jensen. The root-lesion nematodes *Pratylenchus pratensis* (de Man) Filipjev and *P. penetrans* (Cobb) Sher & Allen are known to occur in all major agricultural regions of western Oregon. Exact mapping of their distribution would be useful to nurserymen and others whose crops require nematode-free soil. Soil samples and root samples of host plants from 97 locations in Marion and Polk counties were processed by standard nematological methods. Although *Pratylenchus* spp. were found in 53 per cent of the samples from cultivated areas, only 22 per cent of the permanent pasture samples and 6 per cent from timbered areas were infested. This survey of the 2 counties indicates that tilled and unimproved areas, especially at the higher elevations, have remained comparatively free from these 2 species of *Pratylenchus*.

Red stele of loganberry. McKeen, W. E. Red stele of loganberry is very prevalent in plantations on the Saanich peninsula of Vancouver Island, where it causes decay of a large part of the root system and decline of infected plants. This disease occurs during the cool wet winter season; in many instances, the roots on the "tips," which are especially suitable for etiological studies, are completely destroyed. The large white "tip" roots usually become infected near the growing point, the stele becomes red, and later the whole root turns chocolate brown and disintegrates. A *Phytophthora* sp. is the primary parasite, but it is closely associated with a *Pythium* sp. The *Phytophthora* sp. was quite similar to *P. fragariae* in morphology, in growth-temperature relations of mycelium, in zoospore production, and in inability to grow on media containing reducing carbohydrates. The British Sovereign strawberry is resistant, but the Huxley and Baron Von Solemacher varieties are susceptible. For these reasons, this loganberry *Phytophthora* is considered to be a race of *P. fragariae*.

Verticillium wilt resistance of potatoes correlated with histochemical tests for phenols. McLean, John G., Duane LeTorneau, and James W. Guthrie. Using the ferric chloride test for scab resistance, the authors found scab-susceptible types that reacted to ferric chloride in the periderm and cortex of potato tubers. Highly significant correlations were found between the cortical reaction and Verticillium wilt resistance in the field. Ferric chloride and methyl red were used to test 9 locations in the underground portions of 3 susceptible and 3 resistant potato varieties, from germination to maturity. Highly significant differences were found between resistant and susceptible varieties and between the different test locations. Significant differences were found between tests at different ages of the plants. The decrease in intensity of the color developed in histochemical tests in the root and stem vascular system at the later stages of plant development was correlated with disease development in the field. Susceptible varieties showed a lower total color intensity and more rapid rate of decline in intensity than did the resistant varieties. Paper chromatographic studies have shown that chlorogenic acid occurs in stems, leaves, and roots of potatoes. This compound probably is responsible for the ferric chloride test.

Fusions between primary sporidia from different species of Tilletia. Meiners, J. P., and C. S. Holton. Both positive and negative compatibility relationships were exhibited when primary sporidia from spores of *Tilletia* spp. occurring on wheat and grass hosts were cross mated in culture. Sporidia from spores of *T. controversa* from wheat fused readily with sporidia from *T. guyotiana* and *T. fusca*, and also with sporidia of *T. caries* from *Bromus marginatus* and of *Tilletia* sp. from *Bromus tectorum*. In addition, the sporidia of the following species were intercompatible as indicated by sporidial fusions: *T. caries* from wheat, *T. fusca* from *Festuca* spp., *T. guyotiana* from *Bromus japonicus*, and *Tilletia* sp. from *Bromus tectorum*. Matings between sporidia of *T. controversa* and those of *T. holci*, *T. cerebrina*, *T. asperifolia*, or *T. elymi* and between sporidia of *T. cerebrina* and those of *T. caries*, *T. fusca*, *T. guyotiana*, or *Tilletia* sp. from *Bromus tectorum* gave negative results. There was evidence of different degrees of compatibilty as indicated by percentage of mated pairs that fused and by the further development of mated pairs. In certain cases, percentage of fusions was higher in interspecific matings than in intraspecific matings of primary sporidia. These results indicate that fusion between pairs of primary sporidia of different species of *Tilletia* has potential value as a tool in the study of taxonomic relationships.

Dosage rates and application methods with pentachloronitrobenzene for control of Rhizoctonia and scab in potatoes. Menzies, J. D. In heavily infested fine sandy loam soil of the Yakima valley, pentachloronitrobenzene (PCNB), at 20 lb./acre, controlled stem canker of potatoes caused by *Rhizoctonia solani*. Tuber sclerotia, caused by *R. solani*, and scab, caused by *Streptomyces scabies*, were controlled at rates of 40 lb./acre. There was no difference in effectiveness between discing or rotary tilling as methods of mixing this fungicide in the soil prior to planting. Harrowing the material into the soil surface after planting but before hilling was not effective. The above rates are equivalent to PCNB concentrations in the soil of 10 and 20 ppm, respectively. If such concentrations can be placed only in the stem-stolon-tuber zone of the soil, control can be obtained by lower rates per acre.

Squash as a differential host for strains of stone fruit ringspot viruses. Milbrath, J. A. The stone fruit virus commonly isolated on cucumber from trees known to contain viruses of ringspot, sour cherry yellows, peach stunt, and prune dwarf, either alone or in combination, has been transferred to several varieties of squash. Symptom expression varied from local lesions followed by secondary necrosis to bright golden leaf patterns. The squash variety Buttercup was selected as an experimental plant because bright golden patterns developed when the inoculum was from source plants with sour cherry yellows. Mammoth White Bush Scallop squash was selected as a source plant for maintaining viruses, since many strains became established in this variety and were carried in small dwarf plants with small leaves having high virus titre. Some strains developed distinct local lesions on Hubbard squash. Twenty-five different virus cultures were established in squash. Viruses were obtained by placing buds from the different source trees into peach and then tranferring each from peach to cucumber and then to squash. Inoculations from 7 sources with a sour cherry yellows history caused a bright golden yellow leaf symptom, very similar to the

pattern of sour cherry yellows on Montmorency cherry. Squash inoculated from other stone fruit sources developed local lesions only, mild chlorotic mottle, severe growing-point necrosis, or no symptoms.

Bud abortion—a virus disease of cherry. MILBRATH, J. A., AND H. E. WILLIAMS. A Napoleon sweet cherry in an experimental plot in an orchard at The Dalles, Oregon, suddenly developed a serious virus-like disease. The first year that symptoms appeared, ½ of the tree was affected, and the following year all the branches were abnormal. The leaves were rolled inward and showed an interveinal chlorotic mottle. There was no new terminal growth on affected branches. A well-defined, shallow necrotic ringspot pattern developed on green fruits. The second year after infection, severe leaf- and flower-bud abortion occurred, leaving numerous branches and flower spurs without leaves or flowers. Inoculated Napoleon trees showed similar symptoms, but inoculated Bing trees developed a severe necrotic reaction. One year after inoculation, most of the branches on 8-year-old trees were dead, and severe branch and trunk cankers developed with considerable gum development. Montmorency showed some dieback and very little terminal growth on affected branches. The leaves were small, mottled, and distorted. No effect of the virus has been noted on inoculated peach trees. Only 1 other naturally infected tree has been noted in the area.

The use of Botrytis for the production of sweet table wines. NELSON, K. E., AND MAYNARD A. AMERINE. It was found that the mature harvested fruit of several varieties of grapes became infected with *Botrytis cinerea* if held at 100 per cent relative humidity for 1 day or more after inoculation with spores. Musts with Ballings as high at 47° were obtained if the infected fruit was kept dry for 7–10 days at 68°F. Natural sweet wines from these musts possessed the distinctive Sauterne aroma and bouquet associated with European wines of this type. Wet periods longer than 24 hours at 68°F, during which a film of water was present on the fruit, resulted in berry splitting owing to the imbibed water. Splitting of the berries permitted undesirable microbiological activity, which resulted in moldy flavors and high volatile acid contents of the musts and wines. Very little berry splitting occurred when the grapes were kept dry at 90 per cent relative humidity and 68°F, even after several days, yet satisfactory levels of Botrytis infection were obtained. The length of the drying period necessary to produce high Balling musts was inversely proportional to the vapor pressure density at 68°F or less. At 95°F, the drying rate was slower and the finished wine had a raisin flavor.

The nonutilization of D*-alanine and growth stimulation by* L*-alanine in the nutrition of Phytophthora parasitica.* NEWTON, WM. Barely significant growth was obtained in cultures of *Phytophthora parasitica* containing D-alanine as the sole source of nitrogen, but abundant growth occurred in those containing L-alanine. Growth increased as the level of L-alanine was elevated from 0.2 to 0.8 per cent, although 0.2 per cent represents a greater quantity than was removed by the fungus. The growth of *Verticillium albo-atrum* was supported by D-alanine, although much less efficiently so than by L-alanine.

Symptomatology of mercury injury of wheat seedlings from seed treatment fungicides used for controlling wheat bunt in the Pacific Northwest. PURDY, LAURENCE H. The wheat varieties Brevor, Omar, Red Bobs, and Rio were treated with Ceresan M (7.7 per cent *N*-(ethylmercuri)-*p*-toluenesulfonanilide) and Panogen 15 (2.2 per cent cyano-(methylmercuri)guanidine) at the recommended rates of application for bunt control, i.e. ½ and ¾ oz. per bushel, respectively, and at 1, 2, and 4 oz. per bushel. The treated seed was germinated in the laboratory and greenhouse. Germination and emergence of samples treated with 0, ½, ¾, and 1 oz. per bushel were comparable, and the seedlings were free from mercury injury. At 2 and 4 oz. per bushel, germination was reduced or completely inhibited, depending on the degree of injury. Such injury was evidenced by an enlargement of the primary root close to the root apex. The amount of root involved varied from a small section behind the apex to the whole root. Root formation was completely suppressed in the most severely injured seedlings. The coleoptiles of injured seedlings were thickened and shortened to an almost spherical shape. Delayed emergence of slightly injured seedlings was due to the restriction of primary root development. In these cases, emergence eventually followed the formation of the fourth and fifth primary roots.

Growth of tobacco mosaic virus in lesions produced on Nicotiana glutinosa. RAPPAPORT, I., AND S. G. WILDMAN. Leaves of *N. glutinosa* were inoculated with purified preparations of 3 strains of TMV, common (U1), mild (U2), and Holmes ribgrass (U8). The plants were maintained for the duration of the experiment under controlled conditions of light and temperature. Growth of the lesions and rise in the infectivity of extracts from the lesions were measured and compared for a 5-day period following the first appearance of necrosis. Each virus strain was observed to spread at a characteristic rate with time. U1 grew the most rapidly, U2 was intermediate, whereas U8 spread the least rapidly. During this period of lesion growth, the infectivity of extracts rose in proportion to the necrotic area of tissue involvement. With respect to yield of virus, the slowest growing lesion, that of U8, was observed to contain significantly more virus activity per mm^2 of necrotic tissue than did either of the other 2 strains. The activity yield of virus from U2 lesions was the lowest.

Identity and host relations of the potato late-breaking virus. RAYMER, W. B. A virus causing the late-breaking disease of potatoes in Oregon was recovered in *Nicotiana rustica* L. by grafting and transmitted for the first time by the aster leafhopper (*Macrosteles fascifrons* Stal.) to tomato, to Jimson weed, and to Ladino, red, alsike, and crimson clovers. Symptoms of aster yellows were produced in aster and celery, indicating that the virus is that of western aster yellows. Symptoms in the clovers were stunting, phyllody, and marginal reddening of dwarfed leaves. Infected tomato plants ceased apical growth but produced narrow, retrorsely curled leaves with yellow margins and purple veins. Typical tomato big bud symptoms were not produced. Symptoms similar to those in tomato were at first expressed in Jimson weed, but the plants later developed a bunchy-top condition caused by proliferation of small distorted leaves on shortened stems. On the basis of symptoms in potato, Jimson weed, and the clovers, the potato strain of aster yellows is closely related to the tomato big bud virus. These viruses appear to be distinct, since the aster leafhopper is not known to transmit tomato big bud virus, and aster yellows virus failed to induce symptoms of big bud in tomato.

A new form of the twisted-leaf virus disease of cherries. REEVES, E. L., AND PHILIP W. CHENEY. Twisted leaf of sweet cherry affects Bing severely and Long Stem Bing and Van moderately, whereas Napoleon, Black Giant, Black Republican, Lambert, Spaulding, Ebony, Lamida, and Black Tartarian are symptomless carriers of the causal virus or do not develop symptoms that can be readily recognized. All these varieties, however, except Black Tartarian, develop recognizable symptoms when inoculated with a new virulent form of twisted leaf virus. The source of the inoculum of the new form was an old Lambert tree growing in an orchard in Stevens County, Washington. When graft-inoculated with the new form of the twisted-leaf virus, the varieties Bing, Napoleon, Long Stem Bing, Lambert, Van, and an atypical Black Tartarian showed pronounced symptoms; the varieties Lamida, Ebony, Spaulding, Black Giant, and Black Republican developed moderate symptoms; and typical Black Tartarian from 2 sources carried the virus symptomlessly.

Reaction of 39 varieties of apricots inoculated with material carrying the ring pox virus. REEVES, E. L., AND PHILIP W. CHENEY. Trees of Riland apricot propagated on seedling rootstocks of Royal apricot, Lovell peach, wildgoose plum (*Prunus munsoniana* Wight & Hedr.), chickasaw plum

P. augustifolia Marsh.), and Myrobolan plum (*P. cerasifera* Ehrh.) showed equally pronounced fruit symptoms of ring pox when inoculated by grafting with fruit tissue, spurs, or buds from diseased trees. The following varieties propagated on similarly infected Royal apricot and Lovell peach seedlings showed fruit symptoms of ring pox: Big Boy, Bowers, Brooks, Coe's Selection, Early Moorpark, Golden, Golden Giant, Late Yellow, Moorpark, Newcastle Early, Stella, Tilton, Tuttle's Selection, Wenatchee Moorpark, and Wilson. When similarly propagated and inoculated, the following varieties did not show fruit symptoms of ring pox: Anda, Christensen, Doty, Earle Orange, Early Bee, Early Red, Gilbert, J. L. Budd, Manchu, McKelvie, Reeves, Reliable, Southwick, Starglow, Sunglo, Wilson Delicious, Blackwood Royal, Blenheim (Royal), Chinese, Derby, Newcastle, Royal, and Yakamine. The last 7 varieties proved to be symptomless carriers of ring pox virus.

Phytophthora root rot of native Chamaecyparis lawsoniana. ROTH, L. F., E. J. TRIONE, AND W. H. RUHMANN. For 18 years, white cedar root rot has depleted nurseries and destroyed home plantings of Chamaecyparis in the Pacific Northwest, west of the Cascade Mountains. Losses have been great in populous valley areas but until recently were unknown on the southwest Oregon coast in the relatively isolated native range of Chamaecyparis. In 1952, young trees in Coos Bay were found to be infected, marking the first report of Phytophthora in native Chamaecyparis growing under forest conditions. No further infections were found in 1952, but in June, 1953, 3 more trees were located within 12 miles of the original. Two of these from Millington, Oregon, were new; 1 from a Coos Bay park appeared to antedate the 1952 infection. By autumn, killing of ornamentals was common, and by 1954, infection was widespread in young forests of the area. Damage was most apparent along roadsides, stream banks, lakes, and sloughs. Within 2 years, the disease spread 75 miles along the coastal shelf through the best Chamaecyparis sites. Progress inland toward the remaining virgin forest has been slower. Symptoms on wild and ornamental trees agree except for variations with infection type.

Rusty spot, powdery mildew, and healthy skin of peach fruits compared histologically. SPRAGUE, RODERICK, AND PEGGYBETH FIGARO. Rusty spot, cause unknown, is a buff "mildew-like" lesion on the fruit of certain varieties of peaches. The spots develop abundant buff hairs that eventually become necrotic, rusty brown, and tend to slough off as the epidermis beneath becomes lignified. Paraffin sections disclosed a definite increase in corky or lignified tissue in both the mildewed (*Sphaerotheca pannosa*) and rusty spot material as compared with healthy peach skin. The increase in lignified skin tissue was especially marked in the rusty spot lesions. Outer cells in this material tended to elongate into trichomes. Mycelium and spores, abundant in mildewed lesions, were entirely absent in the rusty spot lesions. Therefore, the rusty spots are not sterile mildew lesions, as has been contended. There was a moderate increase in plastids in the parenchyma cells beneath the rusty spots as compared to pulp of healthy peaches.

The effects of fluorine on the anatomy of Chinese apricot leaves. TRESHOW, M. This study was made to determine the earliest anatomical effects of fluorine on apricot and prune leaves and to detect any damage that might appear prior to the characteristic marginal leaf necrosis. Hand sections of Chinese apricot and Italian prune leaves were made. Cell sizes, turgidity, starch content, and suberization were determined in leaves from a filtered greenhouse and from a greenhouse fumigated with hydrogen fluoride. Necrosis appeared on leaves of trees in the fumigated greenhouse. A narrow necrotic band spread from the leaf serration tips. Cells of the spongy parenchyma up to 1 to 2 mm from the necrotic margin were plasmolyzed to varying degrees. Palisade parenchyma cells adjacent to the necrotic tissue were slightly plasmolyzed. Necrosis of vascular tissue extended 1 to 2 mm beyond the necrotic border. Epidermal cells appeared normal, even those over the necrotic mesophyll. Starch content, cell sizes, and suberization were normal in leaves from both filtered and fumigated greenhouses. These cellular responses to fluorine were observed only after the appearance of macroscopically visible necrosis on Chinese apricot or Italian prune leaves and were limited to a band of cells, under 2 mm wide, adjacent to the necrotic tissue.

Aerial infections of Port Orford cedar caused by Phytophthora lateralis. TRIONE, E. J., AND L. F. ROTH. Root rot of Port Orford cedar (*Chamaecyparis lawsoniana*), caused by *Phytophthora lateralis*, has increased greatly in southwestern Oregon in recent years and is now epiphytotic. In the summer of 1954, foliage infections and branch and stem cankers due to this fungus were observed. Foliage infections usually begin near the tips of branchlets in localized areas near the soil surface. As the disease develops, the infected area becomes wider and spreads up the tree. Both sporangia and zoospores have been observed on infected foliage. Branch and stem cankers are difficult to detect prior to color changes in the associated foliage. Studies on the temperature range of infection show that infection can occur from 2° to 25°C; however, the temperature range of sporangial formation from infected foliage lies in an intermediate position between these extremes. Foliage infections were observed most frequently in the southern part of the epiphytotic area.

A strain of Pseudomonas syringae pathogenic on cultivated blueberry. VAUGHAN, EDWARD K. In physiologic tests with differential media, differences between isolates of *P. syringae* from *Syringa* spp. and isolates of the blueberry canker bacterium were as great as the differences between organisms from the 2 sources. In cross-inoculation tests, both organisms infected both *Syringa* spp. and *Vaccinium australi* L., although the percentage and severity of infections was always greatest with the organism from the same host genus. The blueberry organism did not cause infection on native *Vaccinium* species, on other Ericaceous plants, or on either sweet or sour cherry. In agglutination tests, each organism reacted only with its own antiserum. On the basis of biochemical and physiologic reactions, the organism belongs in the species *P. syringae*, but because of differences in pathogenicity and serologic activity it must be classed as a distinct strain of that species.

Increase in incidence of crown gall on Mazzard cherry following preplanting treatments with organic fungicides. YOUNG, ROY A., AND I. W. DEEP. For several years, dichlone has been used in root-dip treatments to control black mold (caused by *Chalaropsis thielavioides*) on Mazzard cherry. While evaluating chemicals in preplanting dip treatments for crown gall control, it was observed that the incidence of gall on seedling trees that were root pruned, inoculated, then treated in dichlone (2 lb./100 gal.) was consistently much higher than on similar inoculated but untreated seedlings. The percentage of gall in such trees treated with dichlone, with captan, or not treated was 73.6, 46.2, and 32.5, respectively. Tests were conducted to determine whether the effect of dichlone was in 1) stimulating *Agrobacterium tumefaciens* directly, 2) stimulating growth of Mazzard cherry and thus rendering them more susceptible, or 3) eliminating fungi antagonistic to *A. tumefaciens*. Respiration of *A. tumefaciens* was stopped by 100 ppm of dichlone and decreased by 1 ppm. Dichlone treatment of 1-year-old trees did not consistently increase their growth. Immersing roots of inoculated seedlings in a suspension of fungi cultured from cherry roots significantly decreased the incidence of gall as compared to that of the inoculated check or of dichlone- or captan-treated trees. The increased incidence of gall following treatment of cherry seedlings in fungitoxic chemicals with little bactericidal activity apparently is associated with suppression of fungi antagonistic to *A. tumefaciens*.